Classification of oilseed rape visiting insects in relation to the sulphur supply

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1 Introduction

Oilseed rape is grown in cool temperate regions such as Northern Europe, Canada and China. It is grown for its small seeds, which are crushed to separate the oil from the remaining meal. It is ranked the third most important source of vegetable oil after soybean and palm oil providing 14% of the global demand for edible oil (Marazzi, 2003). In the past the oil has been used in the chemical industry as a lamp fuel and as a lubricant, but is now most often used for human consumption in cooking or for the production of food. Because the meal contains approximately 40% protein by weight, it is often blended in animal feed (Lamb, 1989). The world production of oilseed rape increased from 36 million tons in 2003-2004 to 46 million tons in 2004-2005 according to the FAO. In Germany the yield of oilseed rape increased from about 2 t ha^{-1} at the beginning of the sixties to more than 3 t ha^{-1} in recent years (Christen, 2007). It can be assumed that oilseed rape cropping will further increase in the next years because of the increasing use in human nutrition and its alternative use as bio diesel.

Oilseed rape is a crop with a very high sulphur (S) demand because of its high content of S-containing secondary metabolites such as the glucosinolates (Schnug and Haneklaus, 1998). Till 1970, the S demand of oilseed rape was satisfied in an indirect way because of the environmental pollution and the continuously increasing atmospheric SO_2 concentrations. This changed in the beginning of 1980 when clean air acts came into force and atmospheric S depositions decreased drastically within a very short period of time (Bloem *et al*., 2005). Additionally the fertiliser practice changed and less fertilisers were used which contained S as a by-product for example ammoniumsulphate fertilisers were displaced by ammoniumnitrate. Moreover the yields of oilseed rape increased because of achievements in breeding and all these changes led to the problem that S became a major yield limiting factor in oilseed rape production when no additional S was added by fertilisation. Not only the yield but also the quality of oilseed rape is strongly affected by severe S-deficiency (Haneklaus and Schnug, 2005). In addition, S-deficiency causes remarkable symptoms of S-deficiency in oilseed rape, which can also affect visiting insects and by this affect the biodiversity of ecosystems. Most likely the most important symptoms of S-deficiency with respect to oilseed rape visiting insects are the symptoms of the flowers. With S-deficiency the size and shape of the flowers are changed and the colour of the flower is altered from bright yellow to pale yellow with severe S-deficiency (Haneklaus and

Schnug, 2005). Additionally the scent of the flowers is also changed and all this modifications make the flowers less attractive for insects, for example a reduced number of honey bees was counted on S-deficient oilseed rape plants (Haneklaus *et al*., 2005). Moreover, S-deficiency decreases the accumulation of S-containing defence compounds such as glucosinolates (Schnug *et al*., 1995). Thus S-deficient plants are supposed to show a lower resistance against pest and diseases. A broad range of different insects, pests as well as beneficial insects, feed on oilseed rape and all plant parts (root, stem, leaf, flower and pod) are affected. The most important pests of oilseed rape which were investigated in this work are summarised in figure 1.1.

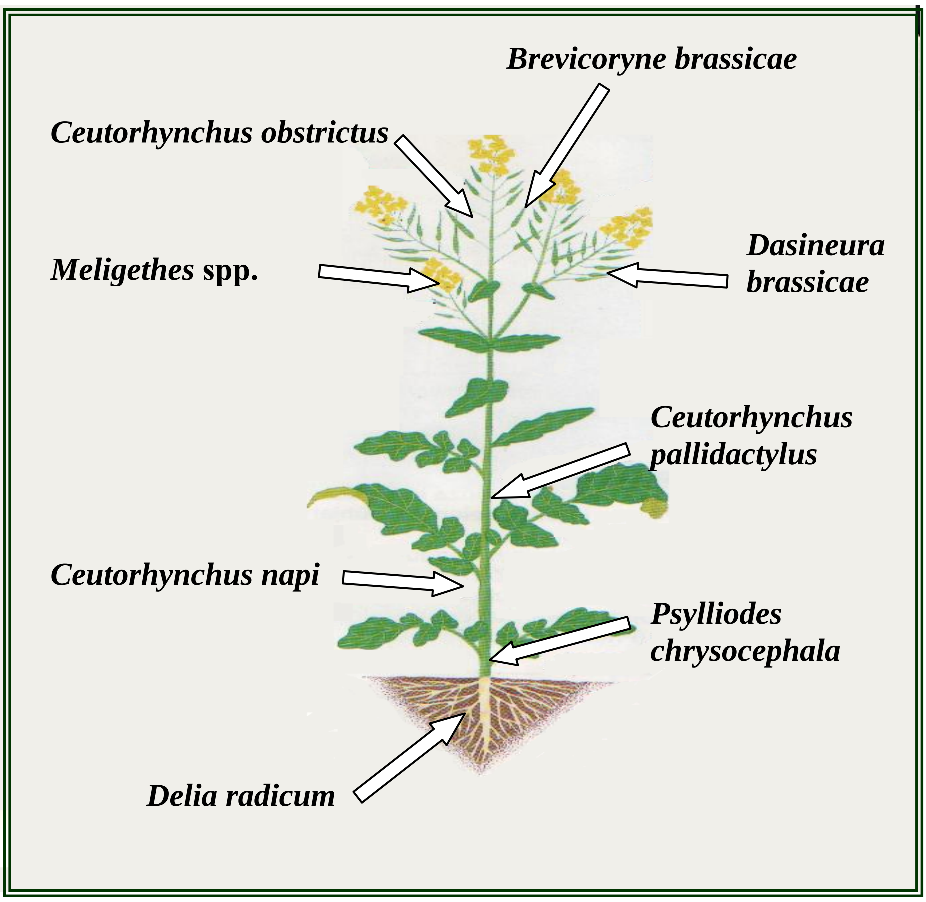
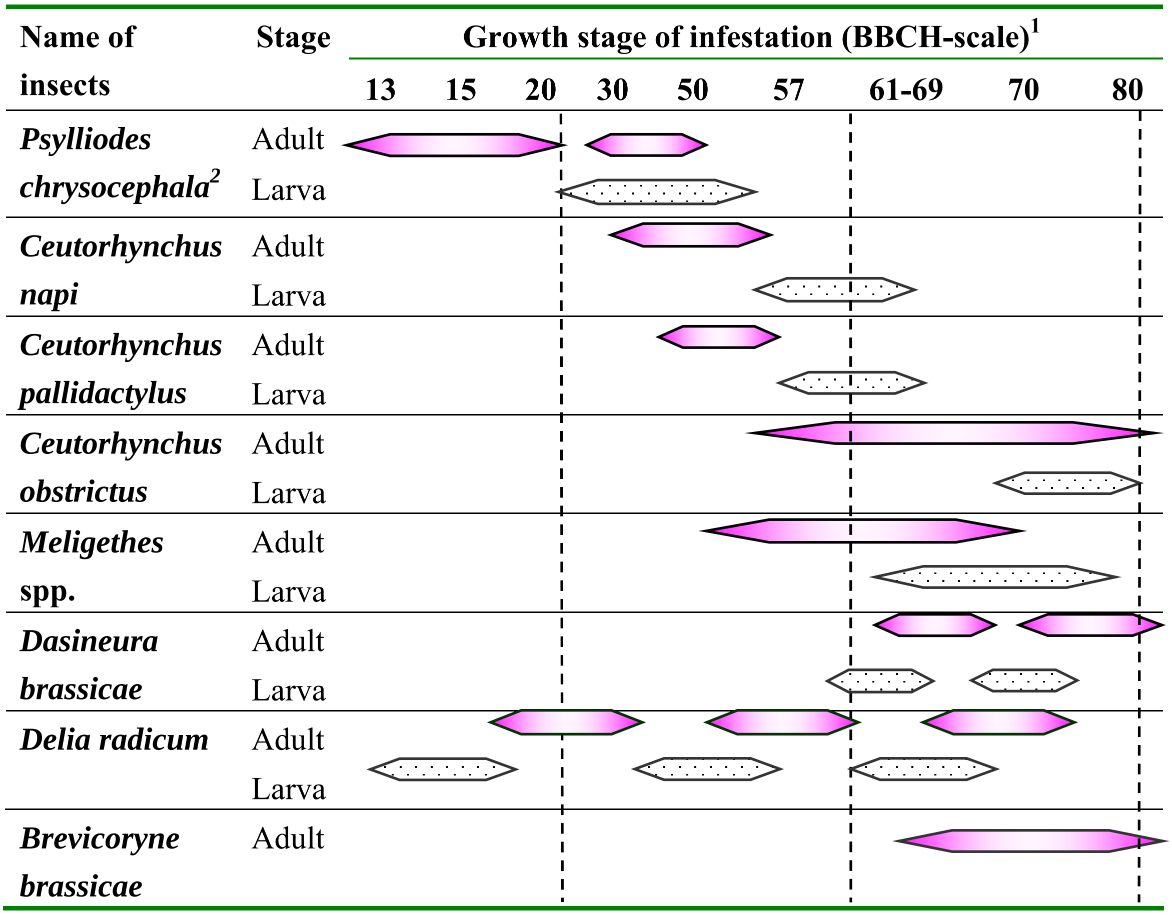


Fig. 1.1: Important pest species which attack different plant parts of oilseed rape.
Source: Büchs and Katzur, 2005; Alford *et al*., 2003; Erichsen and Hünmörder, 2005.

Some pests occur virtually wherever oilseed rape is grown whereas others have a more limited distribution like *Phyllotreta cruciferae*, which is a primary concern in North America, or *Phyllotreta pusilla,* which is associated with oilseed rape cropping in Colorado (Demirel, 2003). Some of them are of special importance for European oilseed rape cropping like the brassica pod midge (*Dasineura brassicae*), while others for example the stem weevil (*Ceutorhynchus picitarsis*) is usually of minor importance (Alford *et al*., 2003). Moreover the damage, which is caused by special insects, can vary for different regions e.g. *Brevicoryne brassicae* cause only minor damage to canola in Colorado while it is a considerable pest in North America (Demirel, 2003). *Psylliodes chrysocephala*, *Meligethes* spp., *Ceutorhynchus pallidactylus*, *Ceutorhynchus napi*, *Ceutorhynchus obstrictus* and *Dasineura brassicae* are the most important pests in German oilseed rape cropping where they can lead to severe yield losses (Büchs and Katzur, 2005). The different insects attack oilseed plants at very different growth stages. In table 1.1 the most important pests in Europe on oilseed rape are summarised together with the growth stage when they affect the plant.

Table 1.1: Important pests of oilseed rape and relevant growth stages for infestation.



Source: Büchs and Katzur, 2005; Erichsen and Hünmörder, 2005; Kirch, 2006.

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[1]: Growth stages according to the BBCH scale of Meier, 2001.
[2]: *Phyllotreta* genus appears at the same time than *Psylliodes chrysocephala*.

1.1 Biology and damage of important pests in European oilseed rape cropping

Oilseed rape can be infested by a lot of different insects. In this chapter the biology of most important insect pests and their damage to oilseed rape were summarised:

Pollen beetle, *Meligethes* spp. (Coleoptera , Nitidulidae)

The *Meligethes* spp. is one of the most important oilseed rape and other cruciferous crops visiting insects in Europe which can cause severe damage and yield losses (Ruther and Thiemann, 1997). This pest is causing great yield losses and high costs for chemical control (Nitzsche and Ulber, 1998). In extreme cases an infestation with *Meligethes* can cause yield losses of up to 50% (Kirch, 2006). Both adults and larvae contribute to economic losses through the destruction of buds and flowers. The adults attack flower racemes to feed on buds, and the larvae feed on the pollen and nectar inside the buds (Blight and Smart, 1999) which results in so called 'blind stalks'. As a compensation reaction the plant is building new racemes and buds which results in an unsynchronised pod filling, a lower seed number per pod and a slightly higher "thousand seed "weight (Alford *et al*., 2003).

After hibernation adult beetles as phytophagous insects feed on the pollen of diverse flowering plants but they lay their eggs exclusively in buds of Brassica crops (Mänd *et al*., 2004). The beetles are able to find their host plants at very early bud-stage by recognising volatiles emitted from the plants (Cook *et al*., 2006). They migrate into winter oilseed rape fields and the females start to lay eggs by biting a small hole into the base of the flower bud and depositing eggs on the stamens or pistil (Fig. 1.2). The eggs hatch within 4 to 9 days and the larvae have two instars over a period of 30 days (Hiiesaar *et al*., 2003). Both larval stages feed on the pollen and nectarines inside the buds. The first instars remain in the flower buds while the second instars migrate to other buds, then drop to the ground to pupate in the soil (Borg and Ekbom, 1996). After two weeks the new adults emerge from the soil, feed on buds and immature green seeds until the end of flowering before entering winter hibernation until next spring (Alford *et al*., 2003).

In the past few years *Meligethes aeneus,* resistant to pyrethroid insecticides has emerged in different European regions such as Germany (Heimbach *et al*., 2007), Denmark (Hansen, 2003), Switzerland (Derron *et al*., 2004) Österreich and southern Sweden (Kazachkova, 2007). This pest caused losses of oilseed rape from 20 to 100% in Germany in 2006 (Heimbach *et al*., 2007) and losses reached to 70 % in Sweden (Kazachkova, 2007).

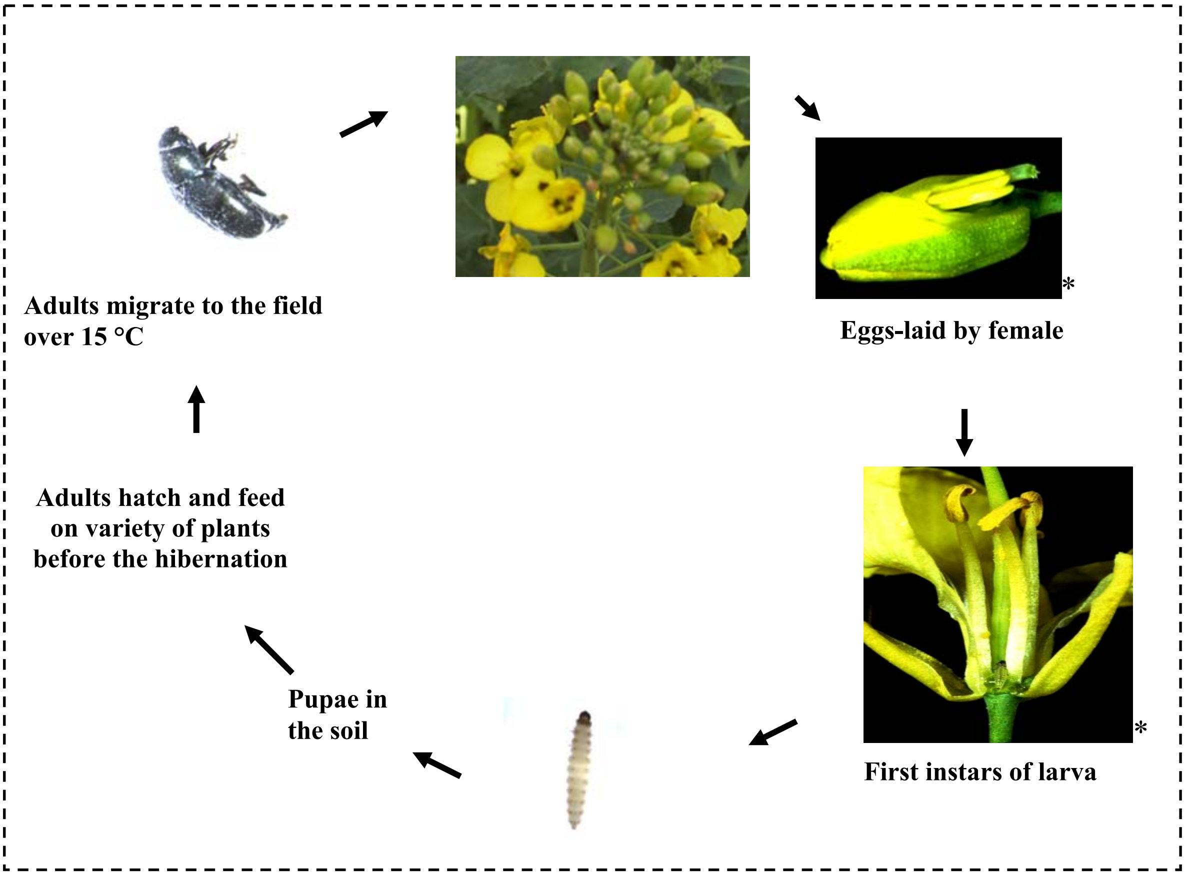


Fig. 1.2: The life cycle of *Meligethes* spp. on oilseed rape (*: the photo from INRA, 2007).

Rape stem beetle, *Ceutorhynchus napi* (Coleoptera, Curculionidae)

Ceutorhynchus napi belongs to the oligophagous insects and is restricted to cruciferous crops. It is one of most important pest of oilseed rape in Europe which is regularly recorded in Germany (Kirch, 2006). It attacks stems of oilseed rape causing significant yield losses of oilseed rape (Dechert and Ulber, 2004). *Ceutorhynchus napi* hibernates as an adult, inside the pupal cocoon, in which it survives on fat reserves accumulated during the development of its larva. It resumes its activity when the soil temperature exceeds 6 °C at a depth of one inch (Bonnemaison, 1965). The infestation usually takes place from March to April when the temperature rise to 10-12 °C whereby oviposition depends on the growth stage of oilseed rape. Female adults deposit their eggs into punctures (about 1 mm long) in the stems just beneath flower buds at the time of maximal stem elongation (Debouzie and Ballanger, 1993). The larvae feed within the pith for three to five weeks causing a bursting of the stems. At the end of the third larval stage, larvae drop to the soil where they pupate. The new generation emerges in late summer and only one generation is developing per year (Kirch, 2006). The typical symptoms of an infestation with *Ceutorhynchus napi* are stem deformations and a splitting of the stems (Debouzie and Ballange, 1993). These deformations and splittings are mainly caused by the introduction of bacteria or chemical substances during oviposition (Lerin, 1993).

Cabbage stem beetle, *Ceutorhynchus pallidactylus* (Coleoptera, Curculionidae)

Ceutorhynchus pallidactylus is major stem-mining insect that attacks stems of oilseed rape causing significant yield losses of oilseed rape (Dechert and Ulber, 2004). The highest damage of *Ceutorhynchus pallidactylus* comes from larval infestation that causes distortion of tissues and loss of vigour. Adults of this pest migrate to oilseed rape plants from their hibernation sited (in hedgerows and woodland) (Ferguson *et al*., 2006) in early spring and lay their eggs in small groups in the leaf petioles from March to May (Barari *et al*., 2005). The deposited eggs hatch after two weeks, the first and second instars of *Ceutorhynchus pallidactylus* larvae infest the lateral shoots of the plants (Barari *et al*., 2005), tunnel inside the leaf petioles and midribs, and then bore into the stems forming extensive galleries, where they are frequently associated with larval instars of *Ceutorhynchus napi* (Dechert and Ulber, 2004). Larval infestations cause a distortion of tissues and loss of vigour. The mature third instars larvae leave the stem through an exit hole then pupate in the soil during July and August. There is one generation per year. This insect is of minor importance in winter rape, but in spring rape it can significantly reduce yield (Alford *et al*., 2003).

Cabbage seed weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae)

Ceutorhynchus obstrictus is one of most important pests of flowering period causing yield losses through damage to the pods (Cook *et al*., 2006). Immature adults of the *Ceutorhynchus obstrictus* emerge from their overwintering sites in spring and migrate to oilseed rape plants during flowering (Alford *et al*., 2003). It needs Brassica crops for feeding and reproduction. Females deposit one egg per pod and the eggs hatch after two weeks. Moreover egg-laying punctures in pods provide entry sites for *Dasineura brassica* (Ferguson *et al*., 1995). Larvae feed on seeds within developing pods, usually wasting about five seeds before becoming full grown (Alford *et al*., 2003; Cook *et al*., 2006). After 2-3 weeks of feeding, the mature third instars bore through the walls of the pods, then emerge from the pods via exit holes, and drop to the ground to pupate. There is one generation annually (Alford *et al*., 2003). The new adults feed on various Brassica plants for some weeks, and later they hibernate in leaf litter on field margins and adjacent woodland.

Larval feeding damage can cause significant economic losses at various stages of crop development (Buntin, 1999). The seed weight per pod was reduced by 18% when pods were infested only by a single larva of *Ceutorhynchus obstrictus* but the reduction in seed weight was as high as 52% when three larvae per pod were counted (Bracken, 1987). Newly emerged adults often feed directly on the seeds. The assessment of yield losses caused by *Ceutorhynchus obstrictus* is complicated because of secondary damages caused by *Dasineura brassica* which use the oviposition holes or wounds on pods from *Ceutorhynchus obstrictus* to deposit their own eggs. The seed yield was reduced by 10-11% when only an infestation with *Ceutorhynchus obstrictus* was observed but increased to 31-34% with a secondary infestation with *Dasineura brassica* (Buntin, 1999).

Brassica pod midge, *Dasineura brassicae* (Diptera: Cecidomyiidae)

Dasineura brassicae is also a serious pest of oilseed rape in many parts of Europe. It is one oilseed rape specialist that infests oilseed rape at flowering and pod setting which is considered the most suitable time for egg-laying (Murchie *et al*., 1997). Adults appear in the early spring. They lay their eggs in batches of 20-30 in the developing pod, often via holes which were formed by other insects such as *Ceutorhynchus obstrictus* (Bracken, 1987). The larvae feed on inner pod walls which lead to a distortion of pods. Midge infested pods are discoloured, bloated, and split open or shatter prematurely to release the full grown larvae (Alford *et al*., 2003). Pupation of the larvae takes place in the soil in 5 cm depth and larvae spin small silken cocoons in which they pupate (Fig. 1.3). Most larvae enter winter diapauses, but some of these larvae immediately pupate, and a new generation of adults appear two weeks later (Alford *et al*., 2003).

Two generations of *Dasineura brassicae* can develop in winter oilseed rape in Germany. The damage of the first generation is often concentrated to the edges of the fields while the damage of the second generation depends very much on the infestation with *Ceutorhynchus obstrictus*, and is very often affecting the whole field (Kirch, 2006). Yield losses of 34% were observed when about 21% of the pods were infestated with *Dasineura brassicae* (Bracken, 1987). Because of the fact that *Dasineura brassicae* can use the oviposition punctures made by *Ceutorhynchus obstrictus* an infestation with *Ceutorhynchus obstrictus* leads to higher infestation levels by *Dasineura brassicae* (Ahman, 1982).

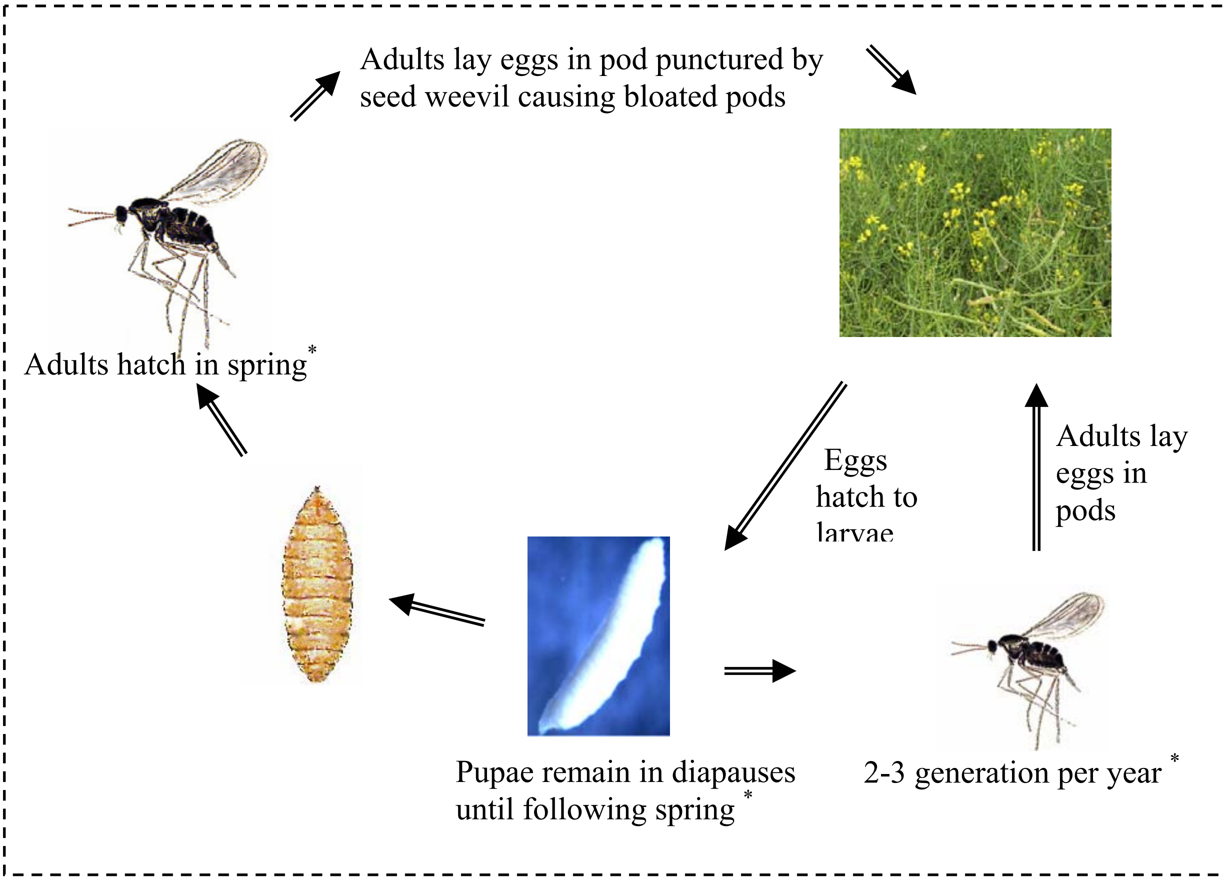


Fig. 1.3: Life cycle of *Dasineura brassicae*. (*: the photo from INRA, 2007).

Cabbage root fly, *Delia radicum* (Diptera, Anthomyiidae)

Delia radicum is a member of a large family of root flies that also includes other pests like seed corn maggot (*Delia platura*) and the turnip root fly (*Delia florilega*) (Jong and Städler, 1999). It is a widely distributed pest of Brassica vegetables including oilseed rape. Only recently *Delia radicum* became an important pest in cruciferous crops in Europe (Erichsen and Hünmörder, 2005). It has been increasing damage in winter oilseed rape in Germany over the past few years (Büchs and Prescher, 2006). Winter oilseed rape is affected by this fly very early in crop development after sowing (Rousse *et al*., 2003). During this time females deposit eggs close to emerging seedlings and larvae invade into roots causing yield reduction (Jong and Städler, 1999). The damaged roots are often invaded by root rot fungi, and the damage of the roots results in significant yield and quality losses. This pest has 3-4 generation in central Europe. The primary symptoms of infestation are roots which start to wilt and becoming stunted (Alford *et al*., 2003; Dorsall *et al*., 2000), and later very often a secondary damage can be observed when root rot fungi invade into the feeding channels which are produced by the larvae (Dorsall *et al*., 2000).

Cabbage aphid, *Brevicoryne brassicae* (Homoptera: Aphididae)

Aphids belong to the most serious pest insect of agricultural crops and they often have specific hosts like *Brevicoryne brassicae* which is a specialist on Crucifers (Nevo and Coll, 2001; Hughes, 1963) but the favoured host is broccoli (*Brassica oleracea*).

Pods of oilseed rape which are affected by *Brevicoryne brassicae* fail to develop properly (Alford *et al*., 2003). *Brevicoryne brassicae* overwinters in the egg stage or, under favourable conditions as wingless aphids. Colonies build up rapidly on infested plants during spring and summer.

Flea Beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae)

The genus *Phyllotreta* is one of the largest and most important groups of flea beetles (Demirel, 2003). It is a well-known pest of all *Brassicaceae* especially vegetable species. It is specialists for glucosinolate-containing families like oilseed rape (Nielsen, 1989). Adults appear in the early spring and feed on cotyledons, newly emerged plants and pitting the leaves causing “pit-like holes” which cause a strong damage and may destroy a plant completely (Hiiesaar *et al*., 2003). The leaf tissue of the cotyledons will die around the feeding site of the adult flea beetles, producing a shot-hole appearance in addition to necrosis (Knodel and Olson, 2002). The feeding damage is greatly enhanced by warm and dry weather in spring, which period the plant is most susceptible to an attack of *Phyllotreta* spp., and can destroy a plant completely. Eggs were deposited in batches in the soil close to a host plant. After eggs hatch, the larvae attack the roots to feed externally. Pupation takes place in the soil and the new generation of adults emerges in late June. There is a single generation annually. Larvae of some species, like *Phyllotreta nemorum*, which frequent occur in Germany, mine within the leaves, petioles of host plants, but they are of minor importance and cause no considerable yield losses in Germany.

Cabbage stem flea beetle, *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae)

Psylliodes chrysocephala is a major pest of winter oilseed rape crops in northern and central Europe. Adults of *Psylliodes chrysocephala* migrate into oilseed rape crops already in autumn where eggs are laid in cracks at the soil surface near the basis of recently emerged oilseed rape plants or at the lower parts of newly emerged plants. Young larvae enter the plants and feed tunnels into stems and petioles (Warner *et al*., 2003).

1.2 Influence of fertilisation on the infestation of crops by insects

Chemical fertilisers are extensively used to increase the productivity of *Cruciferous* crops worldwide (Chen *et al.,* 2004). Past studies have shown that fertilisers have a strong influence on the chemical composition of *Cruciferous* plants and the content of secondary metabolites such as glucosinolates (Schnug and Haneklaus, 1994). The morphology and phenology of the plants is also affected (Marazzi and Städler, 2004; Städler, 1992; Facknath and Lalljee, 2005). Furthermore the leaf surface chemistry and appearance can be influenced by application of fertilisers (Eigenbrode and Pillai, 1998). As a result, fertilisation can affect the susceptibility of plants to insect pests by altering nutrient levels in the plant tissue (Altieri and Nicholls, 2003; Facknath and Lalljee, 2005). However host plant quality and mineral composition are keys determinant of the fertility of herbivorous insects and determines the reproductive strategies of insect such as egg laying, number of eggs, size of eggs, sex ratios, the allocation of resources to eggs and quality of nuptial gifts (Awmack and Leather, 2002). In this chapter the current knowledge about the influence of plant nutrition on pest development is summarised.

The application of nitrogen (N) plays an important role in agricultural production, and the concentration of N in plants can be a limiting factor for herbivorous insects (White, 1993). In about 115 studies crop damage by insect pests increased when the N- content of the host plant was increased (Schoonhoven *et al*., 2005). Higher N-contents in the host plant positively affected the herbivore population (Hugentubler and Renwick, 1995), performance (appearance activity) and density of insects (Chen *et al*., 2004; Facknath and Lalljee, 2005; Bartlet, 1996). Nevo and Coll (2001) could show that the morphology of *Aphis gossypii* was positively affected by N-fertilisation and positively correlated with aphid fecundity. Also, the performance of *Brevicoryne brassicae* decreased with decreasing N supply on Brussels sprout plants (Koritsas and Garsed, 1985). Recently Chen *et al.* (2004) reported that the N-content of leaves of Chinese cabbage was positively correlated with female pupa weight of *Pieris canidia canidia*. It has been also shown that females of the cabbage white butterflies (*Pieris rapae*) prefer to lay eggs on fertilised plants with higher phosphorus (P) and N-contents (Chen *et al*., 2004; Jauset *et al*., 1998). N-fertilisation increased the number of eggs laid by the cabbage butterflies on cabbage and mustard (Hugentubler and Renwick, 1995).

On the other hand low N-contents in host plants resulted in poor larval performance (Chen *et al*., 2004). Some species can compensate low N-concentrations in the plant tissue by a higher nutrient utilisation efficiency such as larvae of *Caligo memnon* and *Obsiphanes* *tamarindi* (Auerbach and Strong, 1981) while other insects compensate low N-concentrations by higher feed intake like the larvae of *Samea multiplicalis* (Wheeler and Halpern, 1999) or by concentrating their feeding on plant parts which contain higher N (Chen *et al*., 2004; Williams *et al*., 1998).

S-deficiency is one of the most widespread nutrient disorders in European oilseed rape production and without S-fertilisation most of the productional fields will show symptoms of severe S-deficiency (Schnug *et al*., 1995). Oilseed rape is particularly sensitive to S-deficiency because of its high demand for S (Haneklaus *et al*., 1999) which is need for the production of seeds and for the synthesis of S-containing phytoalexins and glucosinolates (Dubuis *et al*., 2005). About 16 kg S are required to produce 1 ton of seeds. Additionally S is a structural element of essential amino acids (methionine, cysteine) that are an integral component of full-value proteins (Matula and Zukalovä, 2001).

S-fertilisation enhanced the glucosinolate (GSL)-content in leaves and seeds of oilseed rape (Schnug, 1997; Booth and Walker, 1992; Marazzi and Städler, 2004 and 2005), which was reported to be involved in plant defence against insect pests such as *Papilio polyxenes* (Chew, 1988; Städler, 1992). S-deficiency has been shown to be one of the most common nutrient stress factors, resulting in a loss of crop production, food quality and crop resistance to pests (Schnug and Haneklaus, 1998; Schnug, 1997). With increasing S-supply also plant’s vigour and defence against herbivores is influenced in a positive or negative way (Marazzi and Städler, 2005). There is no consistent opinion about the influence of the S-nutritional status on insects. The S-nutritional status can affect herbivore performance and population dynamics (Bruyn *et al*., 2002) and also the ovipositional behaviour of some species. For example females of the diamond-back moth (*Plutella xylostella*) laid more eggs on S-fertilised plants and the offspring emerging from the eggs (larvae, caterpillars) fed and developed better and faster on S-fertilised plants (Marazzi and Städler, 2004; Marazzi and Städler, 2005). Fertiliser applications do not only affect insect pests but also beneficial insects (Facknath and Lalljee, 2005). Additionally, the S-status of the crop strongly influences the contents of primary and secondary metabolites in the plant material such as the glutathione, cysteine and glucosinolate content in the leaf material (Schnug *et al*., 1995). Glutathione (GSH) for example is a S-containing compound that plays an important role in the interaction of plants with parasitic organisms (Schnug and Sator, 2001).

The nutrients N and S are closely related to each other during the plant growth because they are both used in the protein biosynthesis. The balance between N and S regulates the synthesis of proteins and/or the accumulation of GSL in seeds. A higher S-supply increases the GSL-content of rapeseed (Schnug, 1991) while higher N-concentrations suppress the synthesis of GSLs (Fismes *et al*., 2000). Moreover S-deficiency decreases the efficiency of N-fertilisers (Fismes *et al*., 2000). The poor efficiency of N-fertilisation caused by an insufficient S-supply can lead to high losses of N from cultivated soils (Schnug *et al*., 1993).

Beside of N and S also the content of potassium (K), calcium (Ca), P and magnesium (Mg) in the host plants have a significant effect on insects (Facknath and Lalljee, 2005). All insects require substantial amounts of K, P, and Mg, whereas very little amounts of Ca, sodium (Na) and chloride (Cl) are needed (Dadd, 1973).

The density of aphid populations increased with increasing P-fertilisation (Facknath and Lalljee, 2005). Also, the treatment of barley plants with Mg increased its attractiveness for aphids and aphid reproduction (Havlickova and Smetankova, 1998). K-nutrition plays a role in the resistance of plants to environmental stress, but its effects on insect infestation are contradictorily: the population of European red mite (*Panonychus ulmi*) increased with an increasing level of K-fertilisation on cotton (Facknath and Lalljee, 2005), but the reproduction of aphids was reduced with K-application (Havlickova and Smetankova, 1998).

All this investigation show that the nutritional status of the host plants is of major importance for insect pests as well as beneficial insects and therefore for the biodiversity of agricultural sites. It was the main topic of the present work to investigate the influence of the S-nutritional status of oilseed rape on its fauna under productional conditions. The S-status of oilseed rape is an important yield parameter and very often S is a yield limiting factor in oilseed rape production. Never before the influence of the S-supply on insect pests as well as beneficial insects was investigated in oilseed rape to this extent and the data will deliver insight in the relation between S-nutrition of crops, pest management and biodiversity in agricultural ecosystems.

1.3 Importance of secondary plant metabolites of Brassica napus in host-plant selection

The process by which insects find a suitable plant for feeding or oviposition is usually referred to as "host-plant selection" (Bartlet, 1996). The only tools that plants can use to interact with other organisms are structural elements and chemicals (Schnug and Sator, 2001). The insects are attracted to or repelled by a plant due to a variety of morphological factors such as its shape, size, colour and surface texture (Marazzi, 2003). Moreover, host plant chemistry has an influence on the host-plant selection, and determines the host range of insect herbivores (Chen *et al*., 2004; Thorsteinson, 1960). Host selection parameters can be secondary plant metabolites such as GSL (Bartlet, 1996), allelochemicals (Bernays and Chapman, 1994), volatiles that attract predators or parasitoids (Hilker and Meiners, 2002; Gatehouse, 2002; Birkett *et al*., 2000; Bartlet, 1996) and volatile hydrolysis products (e.g., isothiocyanates) which very often specialists over some distance (Marazzi, 2003). Additionally, GSH is another S-containing compound that plays a role in host selection (Schnug and Sator, 2001).

GSLs and their degradation products are important in the interaction of plants with insects in addition to their role in brassica plant-selection (Hugentubler and Renwick, 1995). GSLs are thought to be advantageous for specialised insects (as attractants, for host-recognition, as defence against enemies, as feeding stimulants and oviposition stimulants) (Schnug and Sator, 2001; Marazzi and Städler, 2004; Bartlet, 1996), but they have on the other hand a deleterious effect on generalist insects (Marazzi, 2003). For example, the egg-laying of *Pieris rapae* adults and larval feeding of *Pieris brassicae* as specialist insects were positively influenced by the GSL-content in plant (Chew, 1988). It was observed that *Pieris rapae* females are stimulated to oviposit by some GSLs (sinalbin, sinigrin, glucotropeolin) (Städler, 1995) and the sensilla on the ventral side of the tarsus contained a receptor sensitive to these three GSLs.

GSLs at the leaf surface can serve as a signal for oviposition for example the cabbage root fly (*Delia radicum*) and the turnip root fly (*Delia florilega*) choose their host plants by recognising GSLs on the leaf surface of host plants (Chew, 1988; Gouinguene and Städler, 2006). These insects receive the chemical oviposition stimuli by the activation of chemoreceptor neurons in sensilla on the ventral side of their tarsi (Roessingh *et al*, 1992), and on the proboscis (Simmonds *et al*., 1994). Only one of four chemoreceptor cells (in sensillum of tarsal) is stimulated by some GSLs (Isidoro *et al*., 1994). Recently Marazzi *et* *al.* (2004) reported that there are two receptor neurons (in tarsal “C” sensilla of adult of *Delia radicum*) sensitive to GSLs.

The volatile breakdown products of GSLs attract specialist insects to their host plants, and the emission of volatile molecules from plants has been recognised as an attractant of pollinators and deterrent of herbivores (Gatehouse, 2002). The pollen beetles and seed weevils orientate themselves to volatiles from oilseed rape using odour-motivated anemotaxis (Bartlet, 1996). *Dasineura brassicae* adults flew up-wind to a field of rape and the females showed a positive anemotaxis in response to rape odour in a wind tunnel (Bartlet, 1996).

Some insects explore host plants before biting or egg laying by using chemical receptors on the antennae, legs and palpe. Adults of *Dasineura brassicae*, for example, walks back and forth on the pod, palpating it with its antennae and mouthpart, and may reject the pod before inserting its ovipositor (Bartlet, 1996). As well butterflies often scratch the surface of potential host plants with tarsal claws before laying eggs (Chew, 1988). So the chemical compounds on the surface of the host plant play an essential role in insect plant-selection (Bartlet, 1996; Marazzi, 2003).

The natural enemies of some important insect pests of oilseed rape are variable in their response to secondary metabolites; some parasitoids are badly affected by allelochemicals sequestered by phytophagous insects from their host plants (Strong *et al.*, 1984). In table 1.2 different compounds are summarised which play an important role in oviposition, selection of host plants and feeding behaviour of the most important oilseed rape visiting insects.

Table 1.2: Compounds which play an essential role in host plant selection for oviposition or which act as feeding stimulants for the most common pests of oilseed rape (*Brassica napus*).

Insect	Plant compounds	Insect stage	Behaviour	Reference
Cabbage root fly *Delia radicum*	Isothiocyanates, volatile breakdown products from GSLs	Gravid females	Oviposition and host selection	Jong and Städler, 1999; Marazzi *et al*., 2004
Cabbage root fly *Delia radium*	Phytoalexins (compounds produced in response to infection or stress)	Gravid females Adult	Oviposition Stimulation	Baur *et al*., 1996; Roessingh *et al*., 1997
Turnip root fly *Delia floralis*	GSL	Gravid females	Oviposition and host selection	Hopkins *et al*., 1997
	GSL	Adult Female adult	Feeding, Oviposition	Baur *et al*., 1996
Diamond back moth *Plutella xylostella*	Indolyl-GSL Allyl-GSL Allyl-GSL	Gravid females Larvae	Oviposition Oviposition Feeding	Städler, 1992
Pieris brassicae	Allyl- GSL	Adult Larvae	Oviposition Feeding	Städler, 1992; Chew, 1988
Pieris rapae	Indolyl-GSLs GSL	Female adult Adult	Oviposition Stimulation	Städler, 1992; Städler *et al*., 1995
Phyllotreta cruciferae	Isothiocyanates	Adult	Host selection	Liblikas *et al*., 2003
Psylliodes spp.	GSL	Adult Larvae	Feeding stimulant	Nielsen, 1989
	Volatile isothiocyanates	Adult	Attractant	Nielsen, 1989
Ceutorhynchus spp.	Volatile isothiocyanates	Adult	Attractant	Bartlet, 1996
Cabbage seed weevil *Ceutorhynchus obstrictus*	Isothiocyanate and volatiles that are metabolites from GSLs	Adult	Feeding stimulant	Bartlet *et al*., 1997
Brassica pod midge *Dasineura brassicae*	Volatiles	Adult	Attractant	Bartlet, 1996
	Allyl-isothiocyanates, GSL	Adult	Arrestant	Städler, 1992
Cabbage aphid *Brevicoryne brassicae*	Isothiocyanate Allyl-GSL	Adult Adult	Feeding Anemotaxis	Städler, 1992; Chew, 1988
Cabbage stem flea beetle *Psylliodes chrysocephala*	GSL Isothiocyanate Isothiocyanate	Adult Female adult Larvae Adult	Feeding Oviposition Feeding stimulant	Bartlet, 1996
Pollen beetle *Meligethes* spp.	Volatiles Odours (leaves, stems, buds) Isothiocyanate	Adult	Host plant location	Bartlet, 1996 Cook *et al*., 2002
Cabbage white butterflies *Pieris rapae*	GSLs	Larvae	Feeding stimulant	Schoonhoven *et al*., 1998
Pieris butterflies	GSLs or their hydrolysis products	Female adult	Host plant location	Chen *et al*., 2004
	GSLs	Larvae	Feeding stimulant	Schoonhoven *et al*., 1998

1.4 Management strategies to reduce the infestation of oilseed rape by insect pests

In nature, plants use many strategies to protect themselves against insects. These strategies can be manifested as antibiosis, where the biology of the pest insect is adversely affected, or as antixenosis, where the plant acts as a poor host and the pest insect choose alternative host plant, or by tolerance to the pest that affords the ability to withstand or recover from insect damage (Smith, 1989). During antibiosis the plant responds to feeding damage of insects by building physical barriers (spines, thorns, surface waxes, trichomes and tough foliage) (Jansson, 2003), chemical defences (toxins, repellents and digestibility reducers with anti-nutritive or anti-digestive components) (Hilker and Meiners, 2002) and allelochemicals as biochemical factors (Facknath and Lalljee, 2005). Plants are also able to respond to oviposition by forming neoplasm and by the production of oviposition deterrents (Hilker and Meiners, 2002). Biochemical factors, which are partly enhanced by fertilisation, are even more important in antibiosis. The emission of volatiles, which attract antagonists of herbivores like predators and parasitoids (Hilker and Meiners, 2002) aims to recruit natural enemies of the pests (Gatehouse, 2002).

The knowledge about natural defence mechanisms of plants against insects can be used to development management strategies. These mechanisms aim to encourage factors that enhance natural resistance to insects and enables a plant to avoid an attack by inhibiting oviposition and feeding, or by reducing insect survival and development.

The nutrient supply is one factor that is affecting plant resistance to insects as well as fungi and may be used to promote effective counter-measures against pests (Salac *et al.*, 2004). The concept of S Induced Resistance (SIR) aims to increase natural components such as H_2S, glutathione, GSLs, phytoalexins (Haneklaus *et al.*, 2002), and the release of S-containing volatiles (Bloem *et al.*, 2004) to combat fungal infections. It was shown that the GSL-content as well as the cysteine and GSH-content and the emission of H_2S could be increased by S-fertilisation (Haneklaus *et al.*, 2007; Bloem *et al.*, 2007). For some of these S-containing compounds it was shown that they also affect insects (Table 1.2) therefore it is important to investigate and quantify the effect of S-fertilisation on infestation of oilseed rape with insects.

For example, two strategies have been proposed for improving plant performance through changing the GSL pattern of oilseed rape and by this pest resistance. The first involves rape lines with low constitutive, but high inducible GSL levels; these would not attract brassica-specialists in the absence of an attack, but would have the potential to protect the plant from generalist feeders. The second strategy involves rape lines with a higher proportion of GSL that do not catabolise into isothiocyanates; the overall GSL concentration of the plant would be maintained as protection from other herbivores, but the plants would be less attractive for specialists (Alford, 2003).

1.5 Response of generalist and specialist insects to defence compounds

The response to plant defence compounds is different between generalist and specialist insects. A specialist herbivore, which is only able to survive on a limited range of host plants, can adopt constitutive detoxification mechanisms for dealing with plant defence compounds. They have the ability to sequester plant secondary compounds, which can simply be stored or metabolised to insect-specific compounds (Larsen *et al*., 1983). They are also able to use them as a defence against their own predators (Gatehouse, 2002). Other insects produce anti-nutritive or anti-digestive components by increased feeding activity or by altering their digestive enzymes in such a way that they become less sensitive towards proteinase inhibitors induced in the plant or towards plant defensive compounds (Hilker and Meiners, 2002). For example when *Phyllotreta cruciferae*, which is a specialist on GSL-containing cruciferous crops, is provided with transgenic *Arabidopsis* plants expressing GSLs at a four times higher level as the only food source, no deleterious effects were observed compared to controls (Gatehouse, 2002). Possible adaptation mechanisms are the rapid excretion of breakdown products from GSL hydrolysis, the hydrolysis of the glucosides, the inhibition of plant GSL hydrolysis, activation of protective enzymes or sequestration of GSLs (Müller *et al*., 2001; Renwick, 2002; Marazzi, 2003). Specialist insects are very often characterized by a distinctive group of allelochemicals (Bowers and Puttick, 1988). These adaptations are energy-consuming processes for the specialist insect, resulting in the retardation of growth and development.

In contrast, generalist insects are predicted to be poisoned or repelled by the chemical defence compounds of plants; they are balancing this disadvantage by using a wide range of plant species as hosts (Bernays and Chapman, 2000).

A different response can be expected from specialist and generalist insects in relation to S-fertilisation as both are differently adapted to plant defence.

1.6 Objectives of this study

In the introduction it was indicated that the nutritional status of agricultural crops can have a strong influence on the composition of pests as well as beneficial insects. S is an important macro-nutrient and there is only limited knowledge about the influence of the S nutritional status on oilseed rape visiting insects. Oilseed rape was chosen as a crop with a very high S-demand because of its high content of S-containing secondary compounds. Besides oilseed rape is a cruciferous crop containing GSLs which made it possible to investigate the influence of the S-nutrition on generalist as well as specialist insects, where different results can be expected with regard to the relation to S. Because of the strong interrelations between S and N the influence of both nutrients on oilseed rape visiting insects were investigated. The key questions of this research work which will be addressed are:

1. Which are the suitable methods to monitor oilseed rape visiting insects?
2. Does the application of S and N have an influence on secondary S-containing compounds in winter oilseed rape (*Brassica napus* L.)?
3. Does the application of S and N affect the composition of oilseed rape visiting insects?
4. Does S-supply have an influence on the relationship between insect pests and their natural enemies?
5. Is it possible to minimise the damage caused by oilseed rape visiting insects by controlling the S-status of the crop?

2 Material and Methods

2.1 Description of study sites

Field experiments with winter oilseed rape were conducted over two years (2003/2004 and 2004/2005) in Braunschweig (E 10° 27`, N 52° 18`). The two fields differed with respect to their location; one trial (2003/2004) was located inside the area of the FAL (Bundesforschungsanstalt für Landwirtschaft) and the other one (2004/2005) was about 2 km away on a site that belongs to the PTB (Physikalisch-Technische Bundesanstalt). In this work the trials are labelled as FAL and PTB. The FAL soil was a loamy sand containing 6.5% clay, 46.5% silt and 47% sand, while the soil of the PTB was a very loamy sand (S14) with 12-17% clay, 10-40% silt and 43-78% sand.

Table 2.1: Description of soil parameters (top soil from 0-30 cm) of investigation sites in Braunschweig.

Soil parameter	**pH**	**N%**	**C%**	**P**	**K**	**Mg**
				mg kg^{-1}		
FAL	6.43	0.09	1.11	179	162	59.8
PTB	5.70	0.15	0.90	99.3	188	38.4
Methods	$CaCl_2$	Kjeldahl	Dry combustion	CAL	CAL	$CaCl_2$
References	Hoffmann, 1991	Schlichting and Blume, 1966		Schüller, 1969	Schüller, 1969	Schacht-schabel, 1954

The FAL field had a higher organic matter content and a higher P and Mg status than the soil of the PTB field (Table 2.1). The FAL site was located inside a forest and the adjacent agricultural area was cropped by winter wheat and maize, while the PTB field was located near a road and close to a settlement with predominantly one-family houses with gardens. The adjacent agricultural area was cropped mainly with winter barley. The previous crop on the FAL site was winter barley while oat was grown on the PTB site. During the whole experimental seasons the climatic data were comparable for both years. The mean temperatures (April-August) for the first and second season were 14.7 °C and 14.8 °C, respectively. The mean sums of sun hours were 1008 h for the first season and 996 h for the second one. The precipitation records in the first season were 247 mm and in the second one 239 mm. The temperature is the most important climatic factor for the development, survival, reproductivity and abundance of herbivores and changes in temperature during different growth stages will affect the development of insects and their appearance time. The changes in temperature and the precipitation rates for the trials are shown in fig. 2.1 and fig. 2.2. The mean temperature between February and March was lower in the second season 2004/2005 compared with 2003/2004.

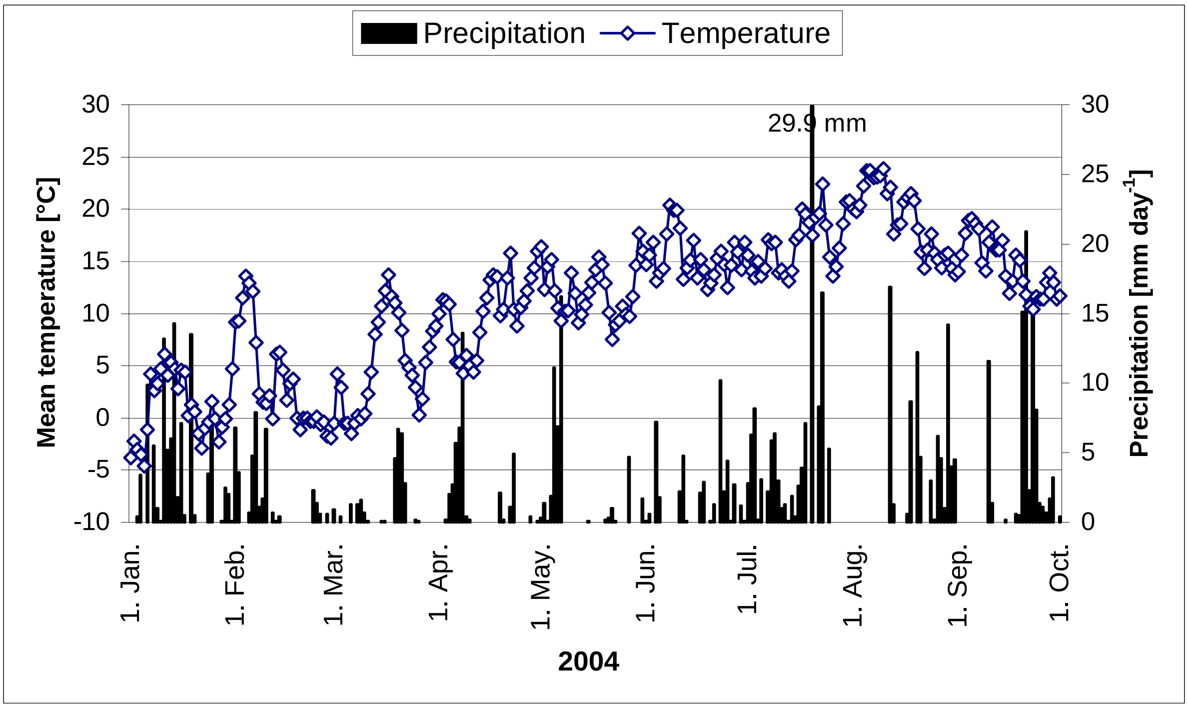


Fig. 2.1: Precipitation and temperature during the growing season 2004.

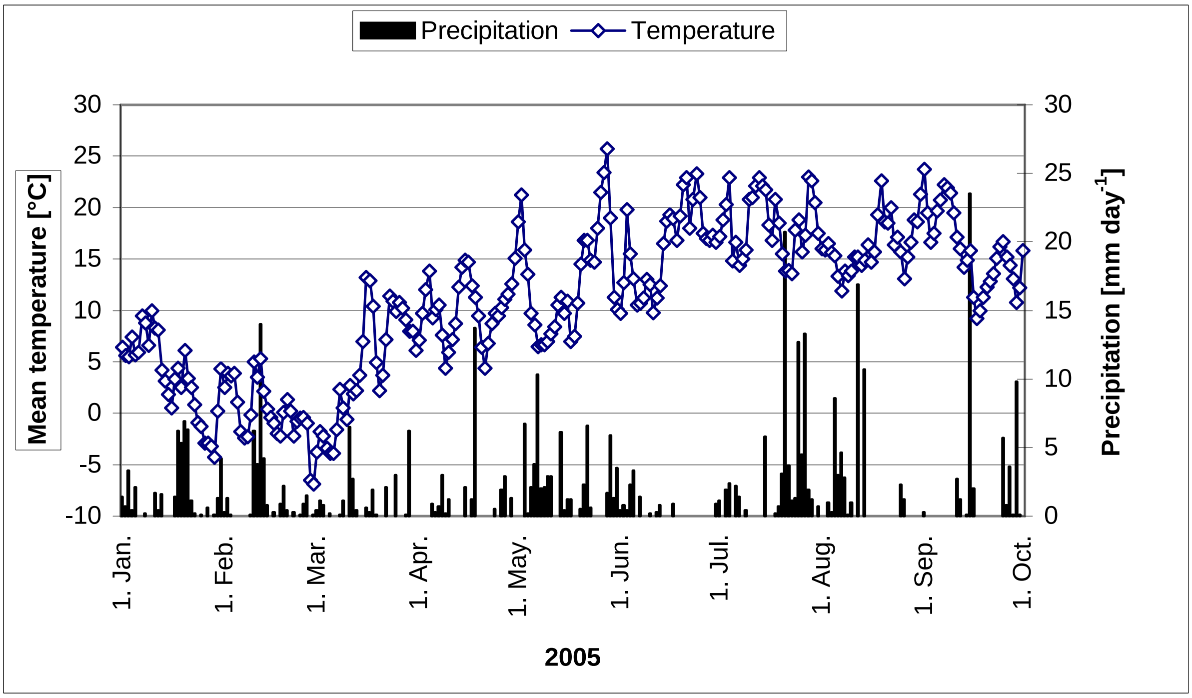


Fig. 2.2: Precipitation and temperature during the growing season 2005.

2.2 Experimental design

In the first season 2004, the focus of the measurement was put on the influence of the S-fertilisation on the number of the most important insects of oilseed rape (adults and larvae). Very different methods were established to catch the different insects at different growth stages. In the following year additionally to the influence of S-fertilisation also the influence of N-fertilisation on the infestation with different insects was monitored (Table 2.2).

Table 2.2: Design of field experiments in Braunschweig (2004, 2005).

Experimental descriptions		**Site**	
		FAL 2004	**PTB 2005**
Investigation parameters	**S rate [kg ha^{-1}]**	0/150	0/150
	N rate [kg ha^{-1}]	200	100/200
Design of the field trial	**Sowing date**	26.08.2003	19.08.2004
	Cultivars	Lipton /Bristol	Lion
	Seed density [kg ha^{-1}]	5	4
	Plot size [m^2]	60	135
Trapping methods		Sweep net, suction trap, emergence traps, funnel traps, plant dissection and yellow water dishes	

The trials were sown in August with a seed density of 5 and 4 kg ha^{-1}, respectively. The first field trial (Fig. A.1) was carried out in 16 plots; the area of each plot was 60 m^2 and each treatment had four replicates. The plots were arranged in a completely randomised block design. 150 kg S ha^{-1} was applied to the soil as potassium sulphate (K_2SO_4), the first rate (75 kg ha^{-1}) was added at sowing date and the remaining 75 kg ha^{-1} were applied in five rates during spring. The control plots received a K balance in form of potassium chloride (KCl). The N-fertilisation of 200 kg N ha^{-1} was applied in form of ammonium nitrate (NH_4NO_3) to all plots in two doses at the start of the vegetative growth (14.04.04 and 31.04.04). Two different oilseed rape varieties (Lipton and Bristol) were grown in the first experiment in 2004 and in 2005 the variety Lion was grown. These varieties differed in their resistance to fungal pathogens: Lipton and Lion were reported to be resistant to *Pyrenopeziza brassicae* and Lion was susceptible to *Leptosphaeria maculans* (HGCA Recommended List WOSR 2003). Bristol was reported to be susceptible to *Pyrenopeziza brassicae* but resistant to *Leptosphaeria maculans* (Gladders *et al*., 1998). For insects no significant differences were reported up to now for different varieties of oilseed rape in Germany (Dechert and Ulber, 2004; Büchi, 1996) but it was no special target of this work to investigate differences in insect infestation in relation to oilseed rape variety. Until now only very limited work is available which investigated the relationship between the occurrence of oilseed rape visiting insects and the variety of oilseed rape.

The second trial was conducted in 16 plots of 135 m^2. The experiment was conducted in two separated blocks with a distance of about 200 m between these two blocks as listed in the appendix (Fig. A.2). The first block was fertilised with 150 kg S ha^{-1} (as K_2SO_4) while the second block received no S-application but a K balance with KCl. S-fertilisation was split in two doses, one was applied at sowing (50 kg ha^{-1} at the 19.08.04) and the second dose was applied in spring (100 kg ha^{-1} at the 07.04.05). N was applied in form of ammonium nitrate (NH_4NO_3) and all plots received 100 kg N ha^{-1} at the beginning of spring (16.03.05). Only the high N-level plots were fertilised a second time in spring (15.04.05) (BBCH 30) with 100 kg ha^{-1} N. Both trials received a well-established pesticide program as it was the main target of this work to investigate the influence of the S-supply on oilseed rape visiting insects under productional conditions. Each plot was treated with 2 L Butisan Top per hectare as herbicide. Insecticides were added two times, the first time was in fall (09.09.04) with 200 ml ha^{-1} of Sumicidin Alfa. The second time was in spring with 200 ml ha^{-1} of Sumicidin Alfa (20.04.05) (BBCH 50) in addition to 75 ml ha^{-1} of Fastac SC (06.04.05) (BBCH 23). Sumicidin Alfa was added to control *Meligethes* spp., *Ceutorhynchus obstrictus*, *Ceutorhynchus pallidactylus*, *Ceutorhynchus napi* and *Phyllotreta* spp. while *Dasineura brassicae* were controlled by applying Fastac SC. The growth stages of winter oilseed rape were determined at every sampling date according to the BBCH scale of Meier (2001).

2.3 Sampling procedures of oilseed rape visiting insects

The different species of insects, which were investigated in this work attack different plant parts at different growth stages. Therefore it was important to use various trapping methods to be sure to sample all different stages of insects, and also to collect flying adult from the plant but also emerging adults from the soil (Fig. 2.3). The plant dissection method was used to determine the percentage of infestation with larvae of the different species. At early flowering the percentage of infestated flower buds with *Meligethes* larvae was determined while *Ceutorhynchus* eggs and larvae were collected from stems at full flowering to study the influence of S-application on the egg-laying by female adults and to determine the severity of infection and percentage of infestated stems. At the beginning of pod development larvae from *Dasineura brassicae* and *Ceutorhynchus obstrictus* were collected from pods to determine the percentage of infested pods.

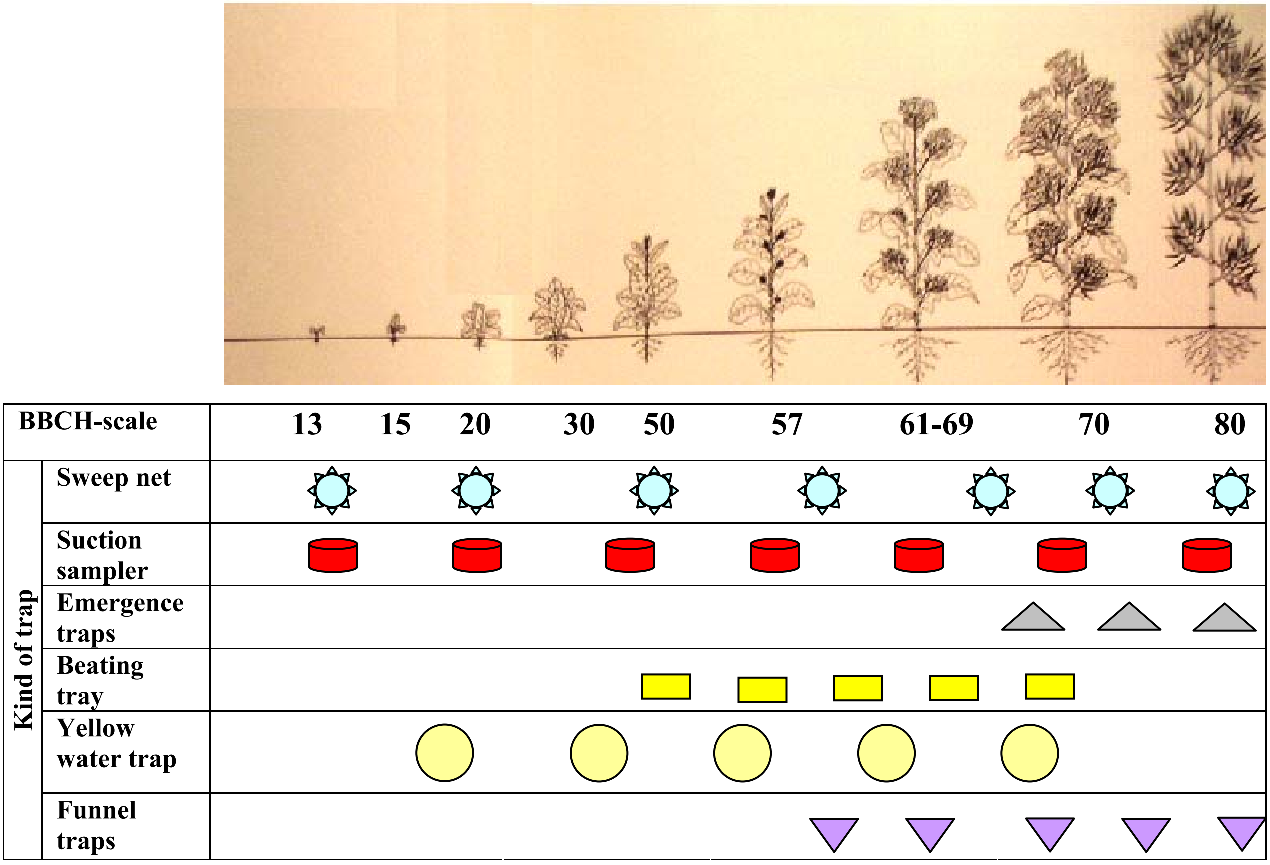


Fig. 2.3: Distribution of traps for the monitoring of oilseed rape visiting insects during different plant growth stages in the field trials.

In the present study very different traps, which are explained more comprehensively in the following chapter, were used for the monitoring and sampling of insects (adults and larvae). The different trapping methods, which are relevant for the collection of larvae and insects, are summarised in table 2.3.

Some methods are more suitable to collect adult insects but also larvae are caught, while some methods are exclusively used for collecting larvae. For example the emergence traps are more suitable to collect adults of *Delia radicum* than the sweep net and the beating tray is the typical method to collect the *Ceutorhynchus obstrictus* and is better suited than the suction trap. The stem-dissection method is exclusively used to sample *Ceutorhynchus pallidactylus* larvae and *Ceutorhynchus napi* larvae while the funnel traps are a typical trap for *Meligethes* spp. and *Dasineura brassicae* larvae. All collected insects and larvae were stored in glass tubes, which contained 70% ethanol prior to classification.

Table 2.3: Methods suitable to collect oilseed rape visiting insects (adults and larvae).

Insects	Method for collecting insects					
	Sweep net	Suction trap	Beating tray	Emergence traps	Funnel traps	Plant dissection
Cabbage aphid (*Brevicoryne brassicae*)	A	A	-	A	-	-
Pollen beetle (*Meligethes* spp.)	A & L	A & L	A & L	A & L	L	L
Rape stem weevil (*Ceutorhynchus napi*)	A	A	A	A	-	L
Cabbage stem weevil (*Ceutorhynchus pallidactylus*)	A	A	A	A	-	L
Cabbage seed weevil (*Ceutorhynchus obstrictus*)	A	A	A	A	-	L
Cabbage flea beetle (*Phyllotreta* spp.)	A	A	-	A	-	L
Cabbage root fly (*Delia radicum*)	A	A	-	A	-	L
Seed corn maggot (*Delia platura*)	A	A	-	A	-	-
Turnip root fly (*Delia florilega*)	A	A	-	A	-	-
Fruit fly (*Scaptomyza flava*)	A	A	-	A	-	-
Brassica pod midge (*Dasineura brassicae*)	A	A	-	A	L	L

A: adult; L: larvae; and A & L: adult and larvae.

2.3.1 Sampling of adult insects

Adults of oilseed rape visiting insects were sampled as they infest or fly within the standing crop by use of sweep net and suction trap or they were sampled while they emerged from pupation in the soil by emergence traps. Some insects were collected when they removed from the crop canopy by using water traps. Other insects such as *Meligethes* spp. and *Ceutorhynchus obstrictus* were sampled directly from infested oilseed rape plants by beating tray. The direct sampling was used particularly when the infestation occurred at an early growth stage of oilseed rape e.g. at green or yellow bud stage or early flowering.

In the following chapters the different trapping methods are described in detail.

I Emergence traps

Emergence traps were widely used in forest ecosystems to record the emergence of canopy insects overwintering in the soil; they were first used in the late 1980s and have been used just recently also in oilseed rape. This method is used for arthropods that emerge from the soil. Emergence traps are related to a defined area and operate continuously; they can provide reliable informations on emergence rates of insects within a defined area and on the origin of recorded arthropods (Büchs, 2003b).

The principle of this trap is based on a positive orientation of the insects to the light. After the insects move to the light, they will arrive in the transparent head box of the trap, which is filled with ethyl glycol as catch liquid. One emergence trap was installed in the middle of each experimental plot at growth stage BBCH 64 (Fig. 2.4) and the head box was sampled weekly. Emergence traps usually cover a circular surface area from 0.25 to 1 m^2 and in the presented trials the traps covered a circular surface of 0.25 m^2. An emergence trap consist of a head box or a transparent sampling vessel at the top and a cone or a dark grey cloth pyramid with an open base that is placed on the soil surface. The top vessel consists of the transparent, removable cover and container where the insects are collected as shown in fig. 2.5. This top vessel is connected to the body of a tent at the tip of the cloth pyramid. The lower inner wall of the plastic tube surface was rough to enable the insects to climb along it into the container. It is important to check the passage between tent and head box regularly for networks of spiders and clean it if necessary.

Fig. 2.4: The sampling of oilseed rape visiting insects using an emergence trap.

Emergence traps are generally used to sample both overwintering insects as well as the new generation of adults of the Coleopterous and Dipterous pests of rape and some of their

Hymenoptera parasitoids as they emerge from pupation in the soils (Williams *et al*., 2003). The species which were collected using this trap in our study were *Phyllotreta* spp., *Ceutorhynchus pallidactylus*, *Ceutorhynchus napi*, *Ceutorhynchus obstrictus*, *Meligethes* spp., *Dasineura brassicae*, *Delia radicum*, *Delia platura,* and *Delia florilega*.

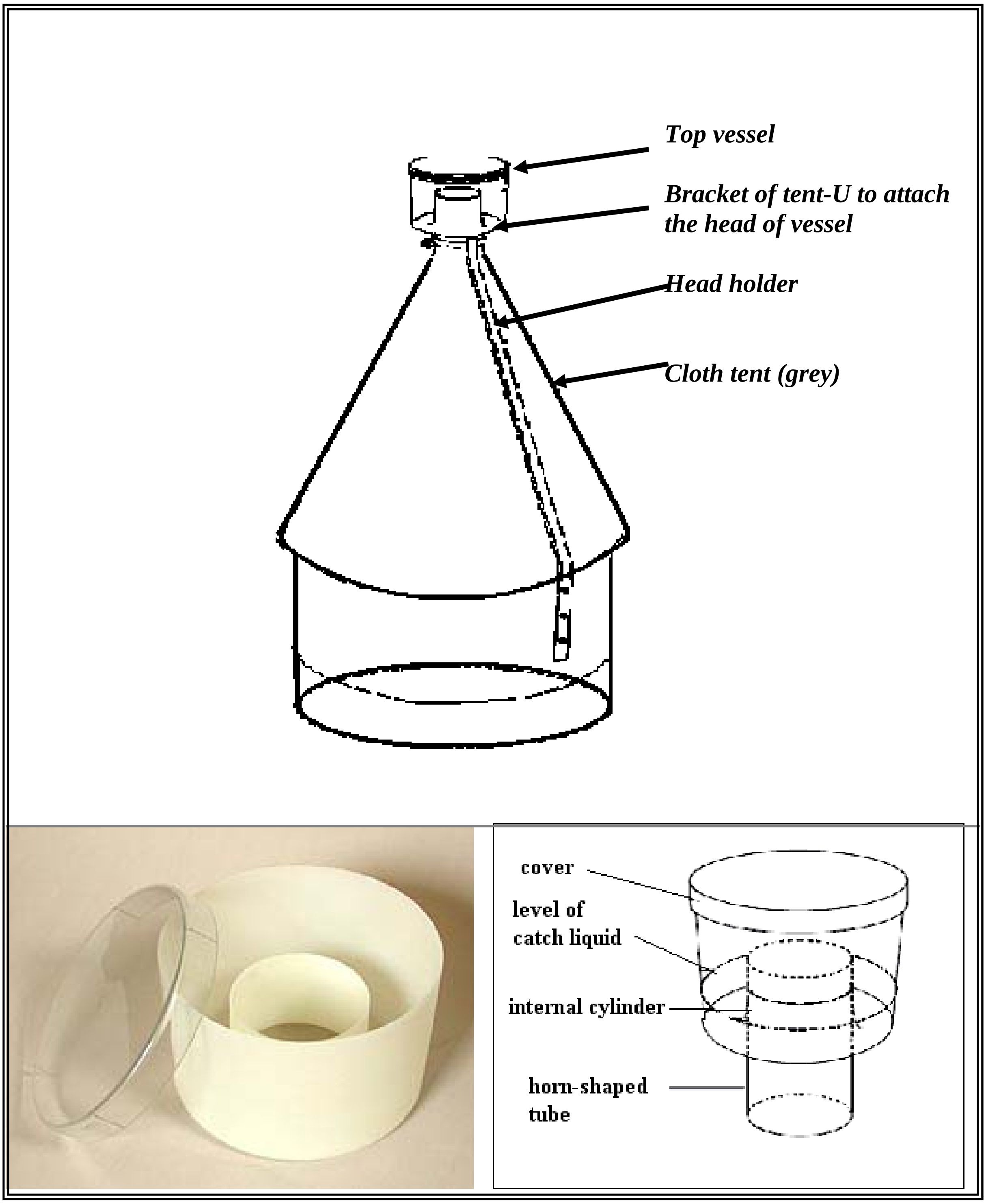


Fig. 2.5: Different parts of un emergence trap (adapted from Nuss, 2004).

II Sweep net

The sweep net is one of the most convenient methods for estimating populations of many different insects. It has been used extensively for sampling most species of oilseed rape visiting insects and their parasitoids. The structure of the hoop-net was a round handle of net bag, which was attached to a woody stick of about 52 cm length. The round handle had a diameter of 30 cm. The net bag was attached to this round handle by a nylon wire and it consists of a very fine crème stainable nettle fabrics of about 60 cm of length.

Sampling was conducted according to the hoop-net method (Witsack, 1975). The first sampling was carried out at BBCH 13 in spring. Generally, the sampling was carried out always at the same time of the day between 12 pm and 2:30 pm. 40 sweeps from each plot are sampled by swinging the net through the plant canopy so that the top of the net was at the same height of the plants. One sweep consists of two hits, one from the left side to the right side and the second is from the right side to left side in an 180° degree arc. After every sweep the net immediately was swung quickly back and forth through the air well above the canopy to force the insects to the bottom of the net. After 40 sweeps, the content of the net was quickly shaken to the bottom of the bag to prevent losses of insects. The caught insects were treated with 2-3 cm large filter papers saturated with ethylacetat, which were put in the net bag before sampling. The insects were carefully separated from reminder of plants before they were put in the storage glass tubes with 70% ethanol. The sweep net method requires the lowest efforts to collect insects but cause some damage to the vegetation and it provides only a relative estimate of insect density. The proportion of caught insects by this method varies in relation to temperature, humidity, wind, altitude of the sun, plant size and length of strokes (William *et al*., 1973). Therefore the sweep net is most efficient when the crops are dry and the weather conditions are sunny with little wind. In this study insects were collected once or twice per week depending on the weather. *Meligethes* spp. and their larvae, *Dasineura brassicae* and their larvae, *C. obstrictus*, *Phyllotreta* spp., *Delia radicum*, and *Syrphidae* were collected by the sweep net method.

III Beating onto tray

Beating tray sampling is an effective method for determining the population of several insects. The main flowering racemes were shaken over a yellow tray and the insects which fall down were collected. The beating tray delivers the best results when the crops are 3 or more inches tall. Therefore, from flowering stage on (BBCH 62), this method was conducted weekly. This method is suitable to monitor insects such as leaf beetles (*Chrysomelidae*), many weevil and Lepidopterous larvae. In the present work, *Ceutorhynchus obstrictus*, *Meligethes* spp. and their larvae were sampled by using this method.

A yellow beating tray is a simple tray of 33 cm * 25 cm * 7.3 cm which was placed below the plants and the plant was hit sharply with a stick (Fig. 2.6). The collected insects were rapidly transferred into glass tubes containing 70% ethanol. This method is a rapid technique and most efficient with sunny weather. The *Meligethes* spp. and *Ceutorhynchus obstrictus* larvae can be counted if they have already left the inside of the plant.

Fig. 2.6: The collection of oilseed rape visiting insects by using a beating tray.

VI Suction trap

Suction trap is used to sample air-borne populations of insects from the above ground parts of plants. The principle of this trap is alike a vacuum cleaner: a sampling vessel is brought above the plant and insects are absorbed. The D-vac suction trap is standard equipment for predator sampling in some regions of Germany (Büchs, 2003b). In this work the vortis suction sampler (Fig. 2.7) was used to collect *Meligethes* spp. and their larvae, *Dasineura brassicae* and their larvae and *Ceutorhynchus obstrictus*. The first sampling as carried out at BBCH 53 and 20 plants per plot were sampled corresponding to an area of about 4 m^2. During sampling it is important to add some drops of detergent to each collection container to reduce surface tension. An advantage of this method is that the collected insects are not injured by the sampling procedure and can be returned to their habitat if this is wanted. The use of suction samplers is limited as the crops need to be dry and moreover this method collects also some organisms from the soil surface. Suction sampling has been used to monitor some of the oilseed rape pest and some of their parasitoids, for example, *Ceutorhynchus pallidactylus*, *Ceutorhynchus napi* and *Psylliodes chrysocephala* (William *et al*., 2003).

Fig. 2.7: The sampling of oilseed rape visiting insects by using the Vortis' suction sampler.

V *Water traps*

Water traps are used for the monitoring of various pests, parasitoids and hover flies (Büchs, 2003b). They are used to sample all of the Coleopterous and Dipterous pests of oilseed rape and some of their parasitoids. Water traps are also suitable to catch insects like *Psylliodes chrysocephala* which move at or near the soil surface. Water traps have been placed into the soil around the field to monitor the immigration of insects and they were dig into the soil so that the upper edges are level with the soil surface (Büchs, 1993). Yellow water traps which were placed on ground level were most effective for catching parasitoids of the *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi*, whereas traps which are installed at the top of the canopy were more effective for trapping parasitoids of *Meligethes* spp.

The height of the trap as well as the colour affects the amount of caught insects. The yellow colour has been long recognised as one of the most effective colours for trapping insects. To sample insects that fly within the canopy, the trap is usually installed in the middle of a plot at the same height as the canopy, and the trap is raised gradually with crop growth. The efficiency to capture *Dasineura brassicae* and *Ceutorhynchus napi* may be increased by adding a GSL-containing extract of oilseed rape to the trapping fluid (William *et al*., 2003). Typically, a water trap is a plastic bowl (210 mm in diameter and 90 mm deep) or rectangular 33.5 cm * 25 cm * 7.3 cm (Fig. 2.8), containing about 1.5 L of water with a few drops of detergent to decrease surface tension and with some sodium benzoate to preserve the insects until the trap is sampled. The yellow traps were sampled weekly and were cleaned after each sampling and filled up with fresh water.

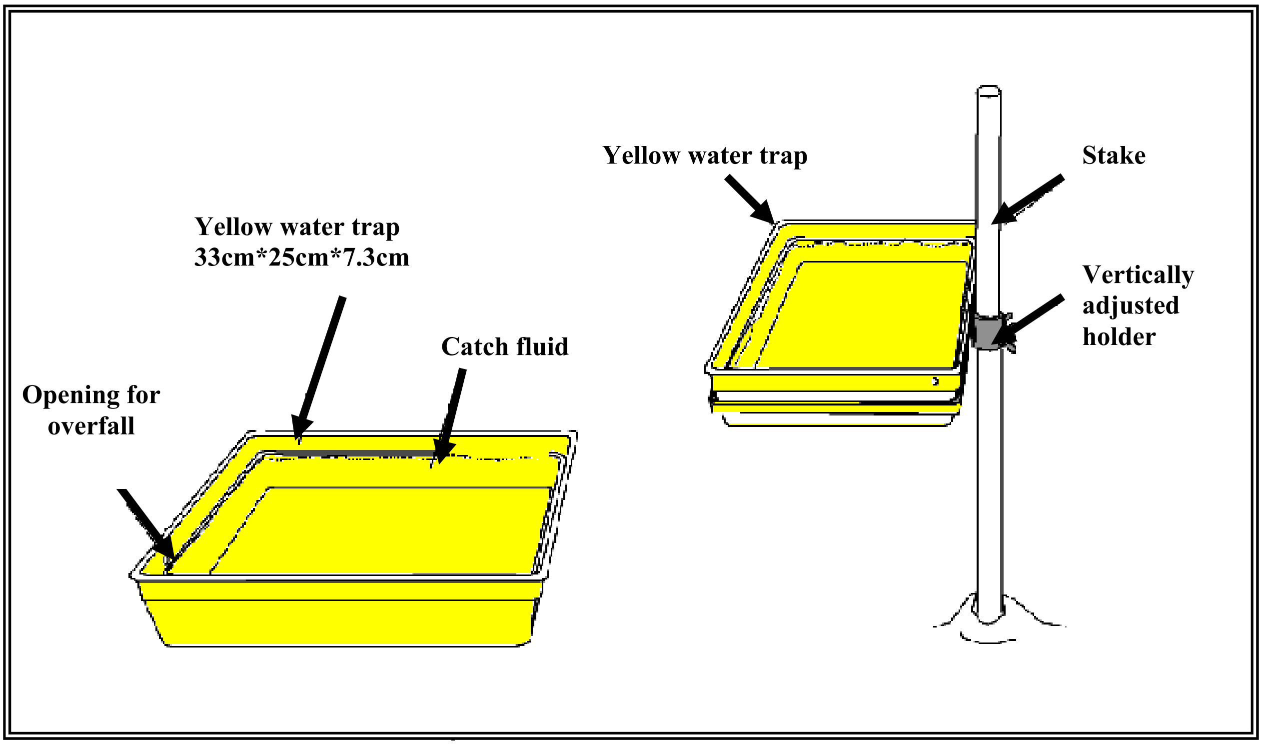


Fig. 2.8: Description of different parts of yellow water traps (adapted from Nuss, 2004).

2.3.2 Sampling of eggs and larvae

Two special methods were used to collect larvae and eggs, the plant dissection method and the funnel traps. As described before some larvae were also collected with other methods like the sweep net, the beating tray and the suction trap. The plant dissection is especially useful to collect larvae in an early stage of development when they are still inside the crops or just before the larvae will leave the plants to migrate to the soil while the funnel traps are suitable to collect full-grown larvae which are ready to pupate.

I Plant dissection

The investigation of eggs and larvae of some oilseed rape pests were conducted by collecting plants from the field and dissecting the relevant plant parts in the laboratory (root, flower, stem and pods). Full-grown larvae should be collected from plant samples just before the start of their migration to the soil; therefore it was very important to determine the suitable time for sampling for each insect (Table. 2.4). The investigation of larvae and eggs was carried out four times in 2004 and five times in 2005. At each sampling, plants were cut off at the root neck then the samples were kept at 4 °C until dissection. The collected individual plants were immediately checked under the binocular to estimate the damage of each plant.

Table 2.4: The different sampling dates and plant parts for plant dissection to investigate the larvae of different oilseed rape visiting insects (BBCH code according to Meier, 2001)

Insect	**Plant part**	**2003/2004**		**2004/2005**	
		Sampling date	**BBCH code**	**Sampling date**	**BBCH code**
Delia radicum	Roots	-	-	23.11.04	14-15
Psylliodes chrysocephala	Petioles	-	-	23.11.04	14-15
Ceutorhynchus pallidactylus ***Ceutorhynchus napi***	Stems	26.04.04	63	10.05.05	65
		18.05.04	67	15.06.05	76
Meligethes spp.	Flower buds	21.04.04	61	01.05.05	62
		26.04.04	63	10.05.05	65
Ceutorhynchus obstrictus ***Dasineura brassicae***	Pods	06.06.04	73	15.06.05	77

At BBCH 14 the feeding damage of the roots by *Delia radicum* larvae was estimated which cause feeding channels in the roots. In the infested roots the percentage of feeding damage produced by these larvae was determined according to Erichsen and Hünmörder (2005) (Table 2.5). In the same plants the leaves and petioles were dissected to determine the population of young larvae of the *Psylliodes chrysocephala* and to estimate the feeding rate.

Table 2.5: Classification of the degree of infested roots by *Delia radicum* larvae according to Erichsen and Hünmörder (2005).

Degree of damage	1	2	3	4	5	6
Percentage of root damage	0	3-14	15-24	25-59	60-90	100

Meligethes spp. feed on pollen and to get the pollen at green and yellow bud stage, they have to chew their way through the buds leading to blind stalks and no flowers will consequently form. The larvae feed on the pollen and nectarines inside the green-yellow buds and the estimation of the feeding damage was carried out during different phases of flowering. In order to determine the percentage of larval feeding damage in the buds, the total number of flower buds, blind stalks and penetrated pod walls were counted. The percentage of infected buds was determined in the following way:

$$\text{Infestation of buds (\%)} = \frac{\text{Number of infested flowers}}{\text{Total numbers of flowers}} * 100$$

Where: infested flowers includes the blind stalks and infected buds.
The determination of the percentage of infestation of buds was made for the main and second raceme separately.

Adults of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* attack the stems of oilseed rape in spring. During stem elongation the larvae of *Ceutorhynchus napi* feed on the pith of stems causing meridian splitting of the main stems, while *Ceutorhynchus pallidactylus* larvae causing severe damage by tunnel into second stems and leaf stalks (Fig. 2.9). Plants were sampled at two different times to monitor the larvae of these two pest species: the first investigation was conducted in April and the second one in May. The number of egg-laying batches and the number of young larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* were determined in the dissected stems during the first estimation (BBCH 65-67). To estimate feeding damage during the second investigation (BBCH 76-79) the length of feeding tubes, total length of plants, number of *Ceutorhynchus* larvae, number of feeding tunnels and emergence holes in the stems were counted. Then the percentage of infected stems was determined in the following way:

$$\text{Infestation of stems (\%)} = \frac{\text{Length of feeding tubes}}{\text{Total length of stems}} * 100$$

The percentage of infestation of stems with larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* were determined for main and second stems separately.

The investigation of oviposition and feeding damage of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* was carried out three times. The first sampling was carried out in April during the main period of oviposition for both species. This sampling was performed to determine numbers of eggs and numbers of egg-batches (oviposition punctures). The second sampling was conducted at end of April to investigate on the first and second instars of larvae while the feeding damage of full grown larvae and their populations per plant were determined in May directly before they migrate to the soil for pupation. The last determination of infection severity with larvae of both species was made based on the number of lesion areas per plant as follows:

1: Low infection (one damaged area per plant)

2: Moderate infection (two separate areas of damage per plant)

3: High infection (three or more separated areas of damage per plant)

Fig. 2.9: A: Meridian splitting in a main stem caused by larvae of *Ceutorhynchus napi*; B: destroyed second stems by larvae of *Ceutorhynchus pallidactylus*.

A further task was to determine the percentage of pods which were infected by the *Dasineura brassicae* and *Ceutorhynchus obstrictus* larvae. During late flowering and pod setting, adults of *Dasineura brassicae* and *Ceutorhynchus obstrictus* attack the pods. Females lay eggs inside the immature pods. *Dasineura brassicae* larva leading to discoloured, distorted and bloated pods (Fig. 2.10), while the *Ceutorhynchus obstrictus* larva causes a reduction in seed yield, seed oil content, seed weight, and seed germination. Larva assessment was performed one time for each year at the pod developmental growth-stage BBCH 73-77. The total number of pods, *Dasineura brassicae*-, *Ceutorhynchus obstrictus* -infected pods and the number of larvae of both species per plant were counted and the percentage of infected pods for both species in the main and second racemes were determined in the following way:

$$\textit{Ceutorhynchus obstrictus}\text{ -infected pods (\%)} = \frac{\textit{C. obstrictus} - \text{infected pods}}{\text{Total pods}} * 100$$

$$\textit{Dasineura brassicae}\text{ -infected pods (\%)} = \frac{\textit{Dasineura brassicae} - \text{infected pods}}{\text{Total pods}} * 100$$

Fig. 2.10: Symptoms on pods infected by larvae of *Dasineura brassicae*.

In this study reproduction success of *Meligethes* spp., was determined as following:

$$\text{Reproduction success of } \textit{Meligethes} \text{ spp.} = \frac{\text{Hatching per m}^2}{\text{Larvae per m}^2}$$

Where: Hatching: denote to number of new generation adults caught by emergence traps.

Larvae: indicate to full grown larvae that collected by funnel traps.

Same calculation was used for *Dasineura brassicae* and *Ceutorhynchus obstrictus* while for *Ceutorhynchus pallidactylus* larvae were calculated in the stems per m^2.

II Funnel traps

Funnel traps were placed below the flowering crop canopy to collect full-grown larvae when they drop down to the ground to pupate. The larvae were collected from flowering stage on but the traps were installed already earlier to avoid damage of the crops. Funnel traps are formed from plastic funnels attached to a pot. The plastic funnel has a diameter ranging from 13.5 to 21cm or has the shape of a square (60 * 60 cm) (Büchs, 2003b). The plastic pot, which was put inside the ground, contained 50 ml of water containing 5% sodium benzoate. In the upper part of the pot, which is attached to the funnel, is a small drainage hole (1mm diameter) to allow rainwater to drain away. Plants were bent together above the funnels and the number of flowering racemes above each funnel was counted to estimate the larval infestation with

Meligethes spp. (Fig. 2.11) (William *et al*., 2003). Full-grown larvae were dropping from plants into the funnels and were collected in the pot. Traps were sampled weekly.

Funnel traps are used to record the dropping of larvae from the flowers or pods of oilseed rape, and so mainly fully fed larvae of the *Meligethes aeneus*, the *Ceutorhynchus obstrictus* and the *Dasineura brassicae* were caught by this trap, in addition to the larvae of predators (Büchs, 2003b). In this study the larvae of *Meligethes* spp., *Ceutorhynchus obstrictus* and *Dasineura brassicae* in addition to *Staphylinidae* and *Tachyporus* larvae as predator were caught by funnel traps.

Fig. 2.11: Sampling of insect larvae from oilseed rape caught by funnel traps.

2.4 Analysis of plant material and larvae

Larvae and plant leaves of these two experiments were analysed for their total S and other mineral nutrient contents. In addition, S-containing secondary compounds (GSLs) were determined in leaf and seed samples as well as the organic S-compounds were determined in leaf samples.

2.4.1 Analysis of plant samples

Leaf samples of oilseed rape were collected at stem elongation and seeds were sampled at maturity. For leaf samples younger fully developed leaves were harvested and divided into two parts, the first one was immediately shock frozen in liquid nitrogen (-196 °C) and stored in a refrigerator at –80°C before freeze drying of the samples. After freeze drying the samples were ground using a coffee mill or mortar. This material was used to determine labile constituents like glucosinolates, cysteine and glutathione which would be degraded during a drying procedure at 60°C.

The second part of samples was dried in a ventilated oven at 60°C until stability of weight and was fine ground using a mill (particles size < 0.12 mm). This sample was used to determine the total content of S, N and other mineral nutrients.

Seed samples were dried in a ventilated oven at 30°C and ground in a coffee mill to determine the GSL-content of the seeds.

1 Mineral nutrients

500-1000 mg of fine ground plant material was digested for 20 minutes in a microwave (S1200 mega) using a mixture of HNO_3 (65%) and H_2O_2 (35%) (4:1) to determine the total S and other mineral content in the leaf material. The samples have to cool down and were diluted with bi-distilled water to a final volume of 25 ml or 50 ml, respectively and afterwards filtered. S, B, Ca, Cu, Fe, Mg, Mn, P and Zn were determined in this sample by inductively coupled plasma-atomic emission spectroscopy (ICP-OES) (Spectro flame M 120 S Equipment).

The standard micro-Kjeldahl method was used to determine the total N in the leaf samples (Schlichting and Blume, 1996)

2 Determination of organic S compounds

Free cysteine, γ-glutamyl-cysteine (γ-gc), glutathione (GSH): HPLC was used to measure the free cysteine, γ-gc and GSH according to Hell and Bergmann (1990). For the extraction of cysteine, γ-glutamyl-cysteine and GSH, 1 ml of 0.1 M HCL containing 4% PVP (Polyvidon-25) was added to 20-30 mg fine ground freeze-dried leaf material. After that the sample was centrifuged to remove the plant debris. Dithiothreitol (DTT) was added to the supernatant in the dark as a reducing agent after 1 h of reduction time the sulphhydryl groups were derivated with 25 µl of 10 mM bromobimane (Sigma No. B-4380). The separation of cysteine, GSH and γ-glutamyl-cysteine was carried out by HPLC using a 250 x 4.6 mm Nova-Pak C18 columns (4 µ) (water 044380). The detection was conducted at 480 nm with fluorescence detection. The chromatograms were used for the identification and quantification of these metabolites in conjunction with calibration curves of the standards.

3 Glucosinolates

The determination of GSLs was conducted according to Rodrigues and Rosa (1999) in leaf material and according to Anon (1990) in seeds.

Determination of GSL in leaf material

The extraction of GSLs from vegetative plant material was carried out in three steps. In the first step 200 mg of grinded plant material was mixed with 3 ml of boiling methanol (90%, v/v) by an ultra-turrax (speed: 20 400 rpm) for 2 minutes. 0.2 ml of glucotropaeolin (1 mg/ml) was added to the samples as an internal standard. The litters from plant were separated by centrifugation for two minutes at 4000 rpm and the supernatant was collected in a 10 ml flask. In the following steps the precipitates were extracted again two times with boiling methanol (70% instead of 90%) and treated with an ultra-turrax just 1 minute. The supernatants were collected together in the 10 ml flask and finally filled up to 10 ml with methanol (70%).

As the methanol would interfere with the HPLC measurement, it was evaporated and the samples were diluted in 2 ml of water and passed through a sephadex column. The GSL-anions were bond to the positive loaded columns while other constituents pass through the columns. Afterwards the columns were rinsed 2 times with 0.5 ml pyridine and finally 75 µl sulphatase were added and the columns react overnight. On the next day 0.5 ml water were added to the columns 3 times to elute the desulpho-GSLs from the columns. These samples were used for the HPLC determination of GSLs.

Determination GSL in the seed samples

0.1 g of fine ground seed material was heated for 1 minute at 75°C then 1 ml of cold methanol (70%) was added to the sample. After 3 minutes a sinigrin-standard was added as internal standard. The sample was heated for 20 minutes. To precipitate proteins 100 µl of lead-barium acetate were added to the sample. 700 µl of this raw extract were added to a DEAE anion-exchange column (DEAE-Sephadex A-25). Finally 75 µl sulphatase were added and the columns react overnight. On the next day 1 ml water was added to the columns 2 times to elute the desulphoglucosinolates from the column.

The HPLC analysis of GSLs was conducted by using a UV detector at 229 nm. The desulpho-GSLs were eluted by a gradient build from acetonitril (20%) and water and two different columns were used for the leaf and seed material. GSLs in leaf samples were separated by a Spherisorb ODS2 column (250 * 4.6 mm, 5 µ) while for the seed samples a HyPersil C18 column (250 * 4.6 mm, 5 µ) was used for the separation. For the quantification the peak area of the internal standard together with the concentration was needed as well as different response factors for each GSL to calculate the concentration of individual GSLs (see Rodrigues and Rosa, 1999).

2.4.2 Analysis of larvae

Preparation of the larvae

Larvae of different species were collected at different growth stages by different methods (Table 2.5). Prior to freeze drying of the larvae the alcohol in which the larvae were stored was evaporated, the number and fresh weight was determined and after freeze drying (Christ-gamma 1-20) also the dry weight was determined. These samples were analysed for their mineral composition.

Determination of the mineral composition of larvae

0.05 – 0.2 g of freeze-dried larval material were digested by a microwave (S1200 mega) in the same way as described for the plant samples. The concentration of B, Fe, S, Zn, P, Mn, Ca, Mg, Cu, was determined in the extracts by ICP-OES. Since the larvae were stored in 70% ethanol there were some precipitates in the alcoholic solution. Therefore the solution was carefully filtered and the deposits were taken up in HNO_3 and given later to the weighed sample material.

Table 2.6: Larvae which were analysed for their mineral nutrients composition by ICP-OES

Name of the larvae	Methods	Growth stages of oilseed rape (BBCH code)
***Meligethes* spp.**	Sweep net	65, 66 and 71
	Suction trap	65, 66, 69 and 72
	Beating tray	66 and 69
	Funnel traps	67, 71 and 72
Dasineura brassicae	Funnel traps	71, 72, 73, 75, 78, 81 and 83.
Ceutorhynchus obstrictus	Funnel traps	73 and 76
Ceutorhynchus napi ***Ceutorhynchus pallidactylus***	Stem dissection	67

2.5 *Classification of insects*

The identification of insects (adult and larvae) was carried out at the BBA (Federal Biological Research Centre for Agriculture and Forestry) depending on morphological keys according to Chinery, 1973; Oldroyd, 1970; Klimaszewski and Watt, 1997; Unwin, 1981; Darvas and Szappanos, 2003; Dosse, 1951; Alford *et al*., 2003. In the present work some of the adults were identified to the species level while others were classified until the genus or family. All the classified insect species are summarised in table 2.7. In addition some insect families were determined which are listed in table 2.8.

Table 2.7: Insects species, which were classified in the present examination.

Trivial name	**Scientific name**	**Family**	**Order**
Cabbage aphid	*Brevicoryne brassica*	Aphididae	Homoptera
Turnip sawfly	*Athalia rosae*	Tenthredinidae	Hymenoptera
Pollen beetle	*Meligethes* spp.	Nitidulidae	Coleoptera
Cabbage flea beetle	*Phyllotreta* spp.	Chrysomelidae	
Leaf beetles	*Lema melanopus*		
Rape stem weevil	*Ceutorhynchus napi*	Curculionidae	
Cabbage stem weevil	*Ceutorhynchus pallidactylus*		
Cabbage seed weevil	*Ceutorhynchus obstrictus*		
Seed weevil	*Ceutorhynchus floralis*		
Sitona beetle	*Sitona* spp.		
Amara ground beetles	*Amara* spp.	Carabidae	
Cabbage root fly	*Delia radicum*	Anthomyiidae	Diptera
Seed corn maggot	*Delia platura*		
Turnip root fly	*Delia florilega*		
Brassica pod midge	*Dasineura brassicae*	Cecidomyiidae	
Leaf miner fly	*Scaptomyza flava*	Drosophilidae	

Table 2.8: Insect families, which were classified in the present examination.

Family	Order
Staphylinidae, Elateridae, Curculionidae	Coleoptera
Sciaridae, Cecidomiidae, Epmidae, Chironomiidae, Bibionidae	Diptera
Formicidae	Hymenoptera

2.6 Statistical analysis

The goal of the present work was to study the influence of S- and N-application on oilseed rape visiting insects. The significance of differences regarding total S, the GSL-content and other elements in S-fertilised and S-unfertilised plants was determined by student's T-test. The distribution of insects depending on N- and S-application was determined using Mann Whitney-U-test. All statistical analysis was performed by SPSS version 12.0 for windows (SPSS Inc., Chicago, IL, USA).

3 Results

The infestation of oilseed rape with different insect pests can significantly decrease the quantity and quality of seed yield. Environmental friendly methods of pest control such as increasing the host plant resistance are of high interest today because consumers are more and more concerned about increasing contamination of foodstuff with remainders of pesticide use. The objective of this work was to evaluate the influence of S-fertilisation on the S-status of the plant as well as the occurrence and extent of oilseed rape visiting insect pests in relation to the S-supply.

The results of the influence of S-fertilisation on S-containing compounds of plants (leaves and seeds) and the mineral composition of larvae of different oilseed rape insect pests are presented in the first two chapters (chapter 3.1 and chapter 3.2). In the following chapters from 3.3 to 3.8 the influence of S- and N-fertilisation on the most important oilseed rape visiting insects is shown. The effect of S-application on predator insects (*Staphylinidae* and *Tachyporus*) and the relationship between the larvae of this predator and larvae of *Meligethes* is presented in chapter 3.9. In the last chapter the effect of S-application on Thrips, *Syrphidae* and spiders is presented (chapter 3.10).

3.1 Influence of S-fertilisation on the S-status of oilseed rape and S-containing secondary metabolites

The measurement of the S-nutritional status of the plants was of high relevance as it is important for this study if the S-nutritional status differed according to the S-supply. The S- and N-content in younger fully developed leaves of oilseed rape as well as the cysteine, the gammaglutamylcysteine, the GSH and the total GSL-content was determined and the results are summarised in table 3.1. In both years the total S-content in leaves of oilseed rape of was significantly higher ($p < 0.01$) with S-fertilisation. In 2004 the S-content increased in medium from 3.9 to 12.4 mg S g^{-1} d.w. with S-fertilised plants. In 2005 the S-content in the control was higher with in medium 6.7 mg S g^{-1} d.w. but nevertheless a significant increase was observed with S-fertilisation. The N-content in the younger leaves of oilseed rape increased only slightly with N-application when no S was fertilised but a significant increase from 39.6 to 52.3 mg N g^{-1} d.w. was observed in plots which were fertilised with S. In the plots which received no S-application the biomass development of the plants was also lower and therefore the lower N-application of 100 kg ha^{-1} was sufficient to accomplish the N-demand of the crop with the lower S-application. With S-application more biomass was build and the demand for N increased, therefore the differences in the S-fertilised plots were more distinctive. The S-containing primary and secondary compounds of oilseed rape also increased with S-fertilisation but this increase was only statistically significant for the cysteine-content of leaves (Table 3.1).

Table 3.1: Effect of S- and N-fertilisation on the mineral composition and primary and secondary S-containing compounds of younger fully developed leaves of oilseed at stem elongation and GSL-content in the seed at maturity.

Variable	Season 2003/2004		Season 2004/2005			
			100 kg N ha $^{-1}$		200 kg N ha $^{-1}$	
S-fertilisation [kg ha^{-1}]	0	150	0	150	0	150
S-content in leaves [mg g^{-1} d.w.]	3.90 a	12.4 b	7.20 a	13.4 b	6.20 a	13.2 b
N-content in leaves [mg g^{-1} d.w.]	63.4 a	58.9 a	52.0 a	39.6 a	54.9 b	52.3 b
Cysteine-content in leaves [µmol g^{-1} d.w.]	0.60 a	1.20 b	No data			
γ-GC-content in leaves [1] [µmol g^{-1} d.w.]	1.00 a	1.50 a	No data			
GSH-content in leaves [2] [µmol g^{-1} d.w.]	25.1 a	31.5 a	No data			
GSL-content in leaves [3] [µmol g^{-1} d.w.]	2.70 a	3.90 a	0.39 a	0.85 b	0.54 a	0.47 a
GSL-content in seeds [4] [µmol g^{-1} d.w.]	3.95	8.72	3.93	7.00	5.48	11.4

[1]: γ-GC-content = Gammaglutamylcysteine-content; [2]: GSH = glutathione; [3, 4]: GSL = glucosinolates, different letters denote significant differences between treatments at $P < 0.05$ by T-test. No statistical test was performed for GSL-content in seeds because $n<3$.

The results clearly revealed that the S-fertilisation was effective in both years in increasing the total S-content as well as primary and secondary S-containing compounds in leaves of oilseed rape. In 2004 severe to latent S-deficiency was observed in the unfertilised plots while in 2005 the S-supply was already sufficient even without S-application. Nevertheless in both years it was possible to investigate the composition of oilseed rape visiting insects in relation to the S-supply as significant differences in the S-supply were determined between S-fertilised and unfertilised plots.

3.2 Influence of S-fertilisation on the mineral composition of larvae collected from oilseed rape

The mineral composition of larvae of different species was evaluated in relation to the S-nutritional status of oilseed rape which differed strongly (Table 3.1, Table A.1, A.2, and A.3). It was assumed that probably a different S-content in the plant will also cause changing S-contents in the feeding larvae.

The S-concentration is shown in relation to the weight of a single larva because the weight differed strongly and is representing different growth stages of the larvae.

***Meligethes* spp.**

In table 3.2 the data for *Meligethes* spp. are shown. The S-nutrition had no effect on the biomass development of *Meligethes* spp. larvae and also no influence on the S-concentration in larvae. The highest S-concentration was found in very young larva with a low biomass.

A close negative correlation was found between the dry matter content of larvae and the S-concentration ($r^2 = -0.95$, $p < 0.01$) (Fig. 3.1). Therefore the S-concentration of *Meligethes* spp. larvae was more affected by the developmental stage of the larva (larval instars) than by the S-concentration of the crops (Table 3.2). It was observed that the S-content in the second instars (collected by funnel traps, at BBCH 72) was lower than in the first instars (collected by sweep net and suction trap at BBCH 65). Young larvae (first instars) had a lower weight but a much higher S-concentration. The expectation that the S-concentration of larvae would reflect the S-content of the crops was not delivered.

S-content in larva collected by suction trap was higher than S-content in larva collected by sweep net (Table 3.2). The reason for this could be that the larvae collected by suction trap have lower weight in comparison with larvae collected by sweep net.

Table 3.2: Biomass and S-content of larvae of *Meligethes* spp. collected by different trapping methods at different growth stages of oilseed rape in relation to S-fertilisation in 2004.

Methods	**BBCH-scale [1]**	**S-fertilisation [kg ha^{-1}]**	**Biomass of larvae**		**S-content [mg S g^{-1} d.w.]**
			Fresh weight [mg larva^{-1}]	**Dry matter [mg larva^{-1}]**	
Sweep net	65	0	0.34	0.08	19.8
		150	0.28	0.09	26.5
	66	0	0.95	0.23	5.50
		150	1.07	0.25	5.07
	67	0	1. 08	0.30	3.50
		150	1.04	0.29	3.78
Beating tray	66	0	0.42	0.09	5.00
		150	0.43	0.09	5.13
	71	0	0.89	0.21	3.24
		150	0.82	0.20	3.38
Funnel traps	67	0	0.91	0.61	4.92
		150	0.76	0.53	5.92
	71	0	1.29	0.27	3.78
		150	1.30	0.30	4.16
	72	0	0.60	0.56	8.23
		150	0.80	0.45	7.27
Suction trap	65	0	0.07	0.03	25.9
		150	0.12	0.05	22.5
	66	0	0.40	0.08	9.86
		150	0.26	0.08	11.2
	69	0	0.70	0.21	5.12
		150	0.76	0.22	4.69
	72	0	0.55	0.22	9.62
		150	0.57	0.17	8.35

No statistical test was performed because n < 3.

1: BBCH scale according to Meier, 2001.

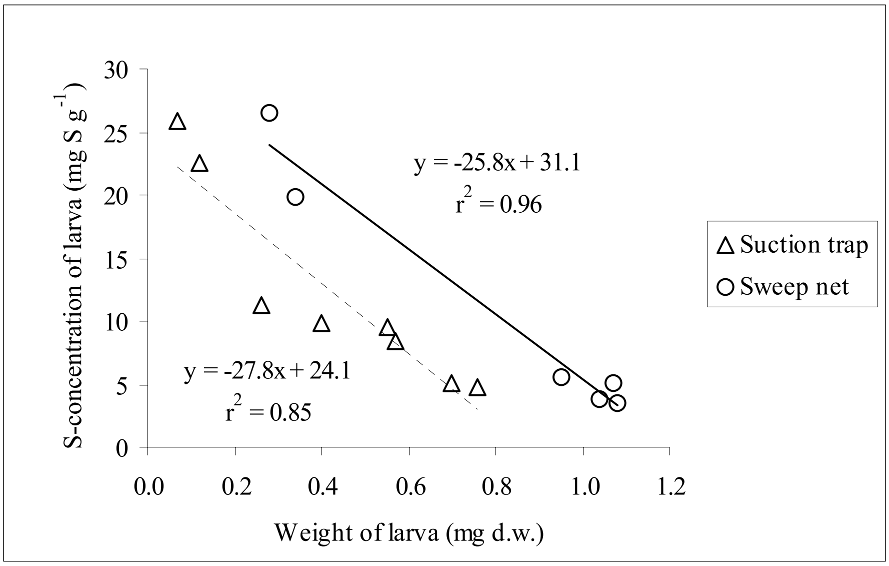


Fig. 3.1: Relationship between the S-concentration and the biomass of larvae of *Meligethes* spp. (larvae were collected by sweep net and suction trap) (season 2003/2004).

Ceutorhynchus napi* and *Ceutorhynchus pallidactylus

Larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* were collected by plant dissection and the fresh weight, dry matter content and S-content were determined in full-grown larvae at BBCH 67 (Table 3.3). The results show that the fresh weight of *Ceutorhynchus napi* was approximately two times higher when collected from S-fertilised plots while no differences were observed for *Ceutorhynchus pallidactylus*. The S-content of *Ceutorhynchus napi* did not differ on a dry weight basis but was very different when expressed as mg S per larva. Slightly higher S-concentrations were also found in larvae of *Ceutorhynchus pallidactylus* which were collected from S-fertilised plots.

Table 3.3: Biomass and S-content of larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* which were collected from oilseed rape by stem dissection in relation to S-fertilisation in 2004.

Larvae species	S-fertilisation [kg S ha^{-1}]	Biomass of larvae		S-content in larvae	
		Fresh weight [mg larva^{-1}]	Dry matter [mg larva^{-1}]	mg S g^{-1} d.w.	µg S larva^{-1}
Ceutorhynchus napi	0	7.42	1.61	3.81	6.24
	150	14.9	2.93	3.85	11.31
Ceutorhynchus pallidactylus	0	4.07	0.88	5.58	4.99
	150	4.06	0.83	6.80	5.72

No statistical test was performed because n<3.

Dasineura brassicae

Larvae of *Dasineura brassicae* which were collected by funnel traps were analysed for their biomass and S-content (Table 3.4). No relationship was observed between the biomass development or the S-content of the larvae and S-fertilisation. The S-content of the larvae was closely correlated with the larval biomass but contrary to the results for *Meligethes* spp. the higher S-contents were measured in bigger larvae and a positive correlation $r^2 = 0.80$ was measured between the fresh weight of larvae and the S-content.

Table 3.4: Biomass and S-content of larvae of *Dasineura brassicae* collected by funnel traps at different growth stages of oilseed rape in relation to S-fertilisation in 2004.

BBCH-scale[1]	S-fertilisation [$kg\ ha^{-1}$]	Biomass of larvae: Fresh weight [$mg\ larva^{-1}$]	Biomass of larvae: Dry matter [$mg\ larva^{-1}$]	S-content [$mg\ S\ g^{-1}$ d.w.]
71	0	0.29	0.10	6.32
	150	0.30	0.12	6.57
72	0	0.29	0.10	6.23
	150	0.33	0.09	4.66
73	0	0.52	0.09	5.87
	150	0.52	0.10	5.77
75	0	2.85	0.13	9.74
	150	3.55	0.11	10.4
78	0	0.20	0.07	4.69
	150	0.34	0.09	4.15
81	0	0.26	0.07	4.26
	150	0.34	0.07	4.96
83	0	0.56	0.03	4.02
	150	0.43	0.02	5.68

No statistical test was performed because n<3.

1: BBCH scale according to Meier, 2001.

Ceutorhynchus obstrictus

The biomass and S-content of larvae of *Ceutorhynchus obstrictus* was also not influenced by S-fertilisation (Table 3.5). The results show no differences in the dry matter content of larvae of *Ceutorhynchus obstrictus* in relation to S-nutrition and only a slightly increase in the fresh weight of larvae collected from S-fertilised plants (Table 3.5). Like in larvae of *Meligethes* spp. the S-content was different between larval stages; the S-concentration was much higher in young larva at BBCH 73 than in mature larva at BBCH 76. The result showed no differences in the S-content of larvae in relation to S-fertilisation when 60% of pods reached their final size, while S-application caused a faint increase in the S-content of larvae at BBCH 73.

Table 3.5: Biomass and S-content of larvae of *Ceutorhynchus obstrictus* collected by funnel traps at two different growth stages of oilseed rape in relation to S-fertilisation in 2004.

BBCH-scale[1]	**S-fertilisation**	**Biomass of larvae**		**S-content**	
	[kg ha^{-1}]	Fresh weight [mg larva^{-1}]	Dry matter [mg larva^{-1}]	[mg S g^{-1} d.w.]	[µg S larva^{-1}]
73	0	10.6	1.90	2.82	5.25
	150	12.3	1.86	3.06	5.75
76	0	13.9	0.85	4.56	3.86
	150	14.5	0.92	4.20	3.90

No statistical test was performed because n<3. [1]: BBCH scale according to Meier, 2001.

Generally, S-application affected the S-content of plant leaves much more than of insect larvae. The results show that on average the S-content in fertilised plants was about 3 times higher than in unfertilised plants (Table 3.1). The S-content of larvae varied only in dependence on the growth stage of the larva and between insect species. The lowest S-content was observed for *Ceutorhynchus obstrictus* with a value around 3 mg g^{-1} d.w. and for *Ceutorhynchus napi* with a S-content below 4 mg g^{-1} d.w. The highest S-content was measured in larvae of *Meligethes* spp. with values as high as 26 mg g^{-1} d.w. at full flowering (see appendix from table A.1 to table A.3). The S-content of larvae of most pest species of oilseed rape was not influenced by the S-content of the host plant but only by the growth stage of the larva. Therefore the results were not consistent with the expectation that higher S-contents in the plant material increase the S-content of the larvae which were collected from that plant.

Beside of the S-content also the mineral concentration of B, Ca, Cu, Fe, Mg, Mn, P and Zn were determined in the larvae and the results are summarised in the appendix (Table A.1, A.2, A.3). The magnesium content was different between larvae species during different growth stages of plants. The highest Mg content was found in larvae of *Meligethes* spp. at full flowering (from 10.6 to 16.0 mg g^{-1} d.w.). It was remarkable that the concentration of microelements (Fe, Mn, Zn, and B) was much higher in larvae than in the plant, e.g. the Zn content in most larvae species ranged from 200 to 250 mg kg^{-1} d. w. Also the Zn content changed with larval growth and for example in larvae of *Meligethes* spp. Zn content increased from 140 mg kg^{-1} d. w. at end of flowering to 590 mg kg^{-1} d. w. in S-fertilised plants at main flowering.

3.3 *Influence of S-fertilisation on the number of pollen beetle (Meligethes* spp.*)*

I Effect of S- and N-fertilisation on the infestation of oilseed rape with adults of Meligethes spp.

In order to investigate the influence of S-supply on adults of *Meligethes* spp., different traps were used to collect adults of *Meligethes* spp. (see material and method). The results from 2004 indicate that S-fertilisation decreased the infestation level by *Meligethes* spp. at an early bud stage when the plants are most susceptible for an attack. When the flower buds begin to rise above the youngest leaves (BBCH 53) and flowers on the main raceme start to open (BBCH 61, 62, 63) an increasing S-supply decreased the relative infestation rate with adults of *Meligethes* spp. (Table A.4) and also larvae (Table A.5). In contrast S-fertilisation significantly increased the number of adult pollen beetles which were collected by sweep net when 40% of flowers in the main raceme were open (BBCH 64) as well as at full flowering (BBCH 66) (Fig. 3.2).

The results obtained in 2005 revealed that not only the S-nutrition but also the N-nutrition had an effect on the infestation level with adults of *Meligethes* spp. at early bud stages (BBCH 53, 62, 64). S-fertilisation decrease the infestation rate with *Meligethes* spp. at early spring from 11% to 25% (Table A.4). At main flowering (BBCH 66) significantly more adults of *Meligethes* spp. were collected in plants that were fertilised with S (Fig. 3.3 and Fig. A.5) and N-fertilisation caused an even higher increase.

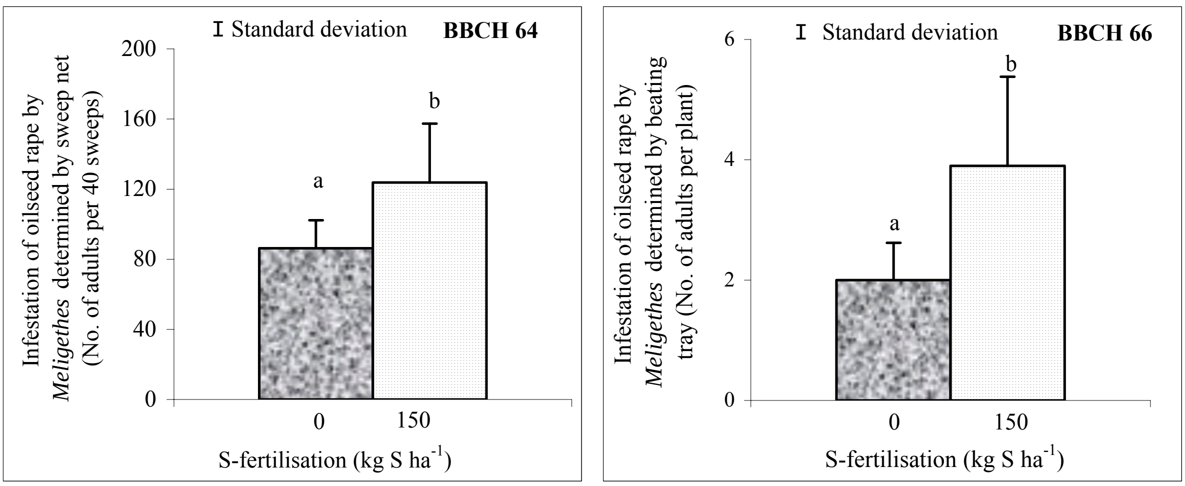


Fig. 3.2: Effect of S-application on the number of adults of *Meligethes* spp. collected by different methods from oilseed rape (var. Lipton) in 2004 at flowering (different letters denote significant differences between treatments at the 0.05 level by the U-test).

Significantly more adults of the new generation of *Meligethes* spp. were collected in S-fertilised plants in 2004 (see appendix Table A.7). Additionally, at full flowering (BBCH 66) and when 60% of pods reached their final size (BBCH 76) a significantly higher number of adults were collected by sweep net and also by emergence traps at BBCH 83 in plots which received the higher dose of N-fertilisation in 2005 (Fig. 3.4).

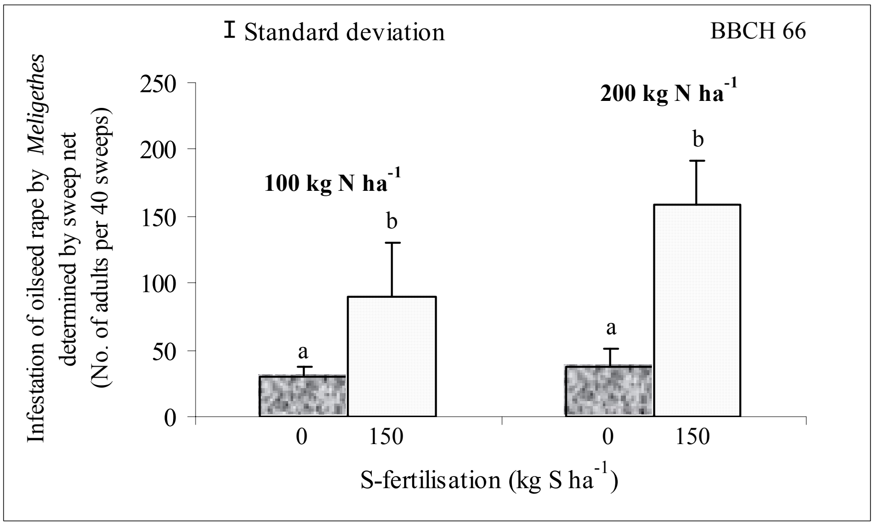


Fig. 3.3: Effect of S-fertilisation on the number of adult of *Meligethes* spp. at flowering collected from oilseed rape (Lion var.) (adults collected by sweep net under N-application in 2005) (different letters denote to significant differences between S-treatments at the 0.05 level by the U-test).

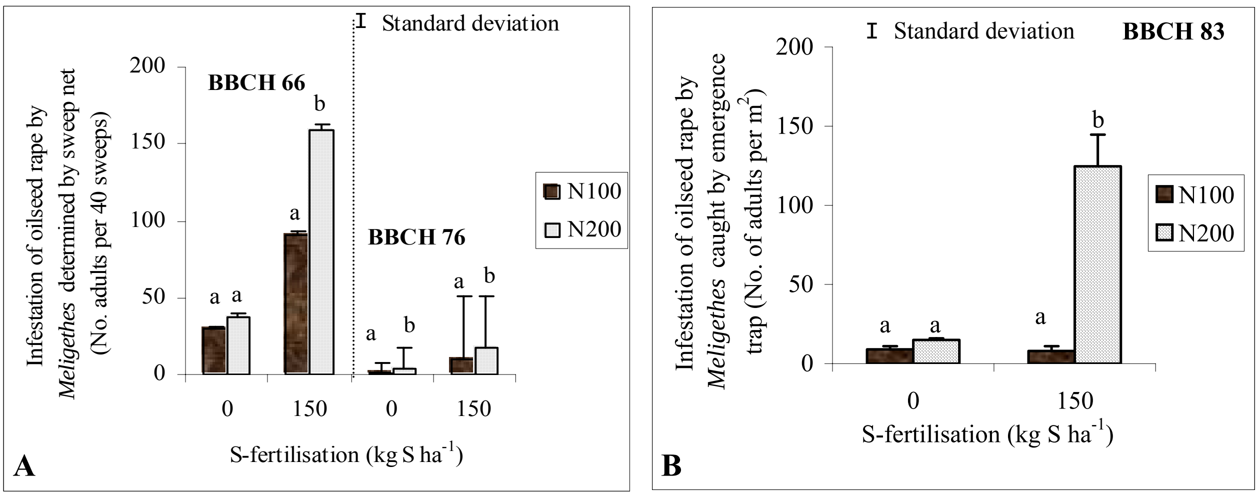


Fig. 3.4: Effect of N-application on the number of adults of *Meligethes* spp. collected from oilseed rape (Lion var.) (adults caught by sweep net (A) and emergence traps (B) in 2005 under S-supply) (different letters denote significant differences between N-treatments at the 0.05 level by the U-test).

A clear interaction between N and S could be observed with respect to the number of adults of *Meligethes* spp. collected at later growth stages. The highest infestation with adults was observed on plants that were fertilised with S and received 200 kg N ha^{-1} (Fig. 3.4, more supporting results are in the appendix Fig. A.5).

This study indicated that reproduction success of *Meligethes* spp. was affected by S-supply and was decreased by about 19% by S-fertilisation (from 0.21 to 0.17).

II Effect of S- and N-fertilisation on the infestation with Meligethes spp. larva

The larval damage was determined at early bud stages by dissecting buds. The sweep net, suction trap and beating tray were also used to collect first instars (young larvae) while second instars (full grown larvae) were collected by funnel traps (see material and methods)

The results indicated that more buds were infected by *Meligethes* spp. when no S was fertilised (Table A.9). Later in the flowering season (BBCH 66) significant more larvae of *Meligethes* spp. were collected by sweep net, funnel traps (Fig. 3.5) and beating tray (Fig. A.6) in S-fertilised plants of the variety Lipton. Also, at the end of flowering (BBCH 69) the number of larvae of *Meligethes* spp. which were collected by suction trap was clearly higher in S-fertilised plants compared to S-unfertilised plants (Table A. 10).

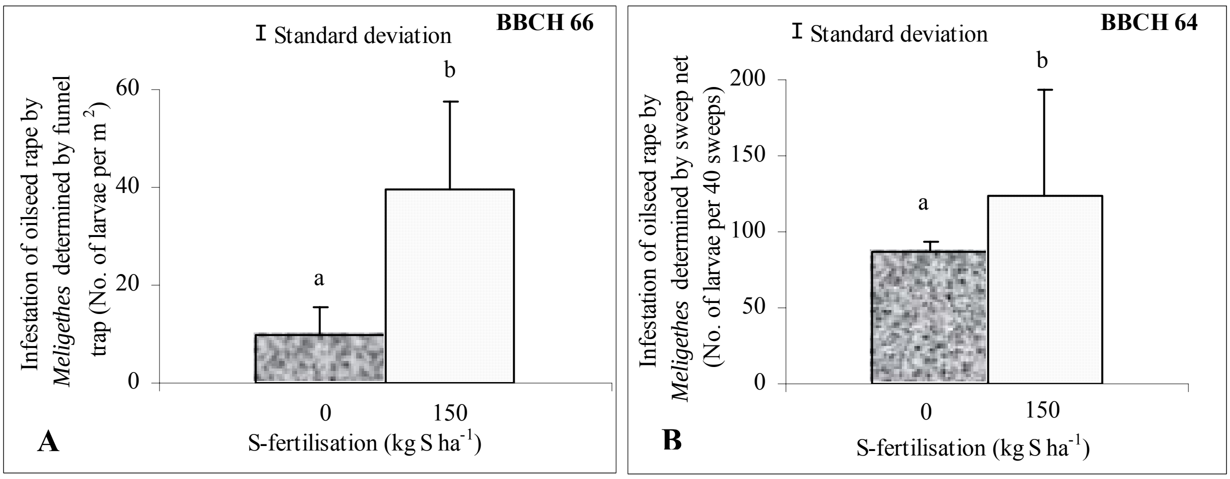


Fig. 3.5: Number of *Meligethes* spp. larvae which were collected from oilseed rape (Lipton var.) by funnel traps (A) and sweep net (B) in relation to S-fertilisation at BBCH 66 in 2004 (different letters denote significant differences between S-treatments at the 0.05 level by the U-test).

In 2005 the effect of S- and N-fertilisation on the infection with *Meligethes* spp. larvae was studied. The results indicated that the effect of S-application on *Meligethes* spp.

larvae was different depending on the growth stage of the plant. S-fertilisation decreased the number of collected first instars of *Meligethes* spp. at early growth stages while higher numbers of second instars were collected from S-fertilised plants from late pod development until harvest. On BBCH 62 and 63, the infection rate of buds was significantly higher in S-unfertilised plants compared to S-fertilised plants (Fig. 3.6). Also, at BBCH 64, 66, 71, 72 it was observed that significantly higher numbers of larvae were collected from S-unfertilised plants by sweep net (Table A.11) and funnel traps (Table A.12). Later when 50% of the pods reached their final size (BBCH 75) the opposite was observed and a significantly higher number of *Meligethes* spp. larvae were collected in S-fertilised plots (Table A.5).

The larvae of *Meligethes* spp. seem to respond positively to N-nutrition (Fig. 3.6, Table A.11, A.12, and A.13). At most growth stages of oilseed rape the infestation with larvae of *Meligethes* spp. was significantly higher in the plots which received 200 kg N ha^{-1}.

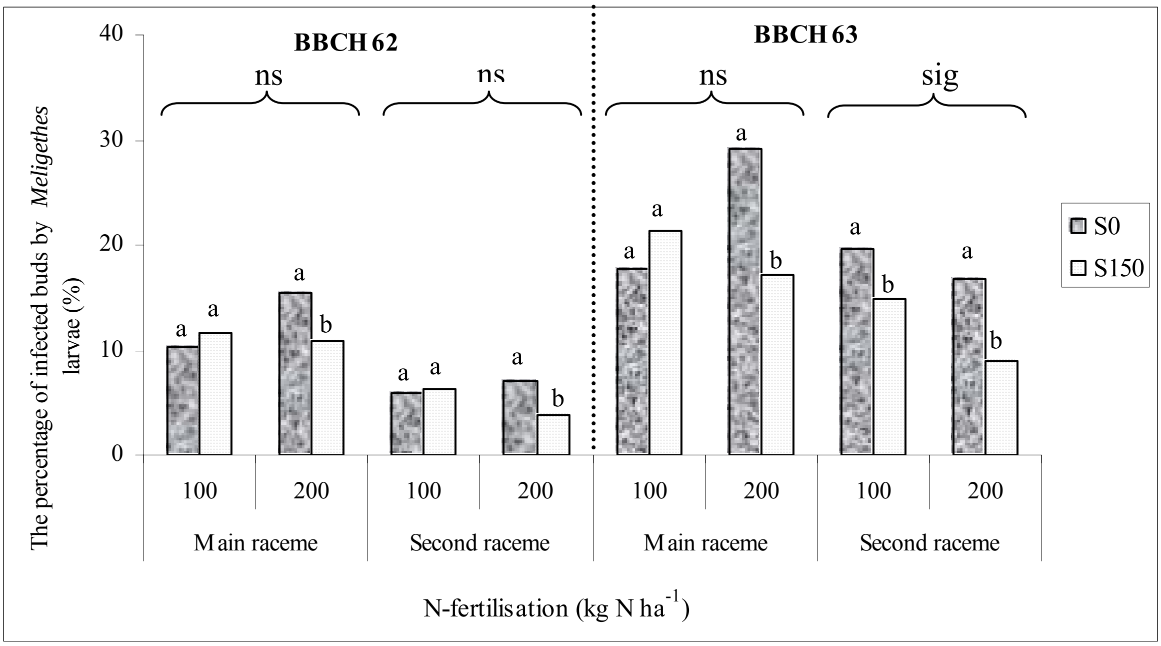


Fig. 3.6: Influence of S- and N-nutrition on the percentage of buds of oilseed rape (Lion var.) which were infested by *Meligethes* spp. larvae at flowering (different letters denotes to significant differences between S-treatments while sig. denotes to significant differences between N-treatment at the 0.05 level by the U-test) (season 2004/2005).

In general the application of S-fertilisers could decrease the damage of *Meligethes* spp. by decreasing the number of adults and larvae at early flowering stage (Fig. 3.7), which is the most susceptible stage to *Meligethes* spp..

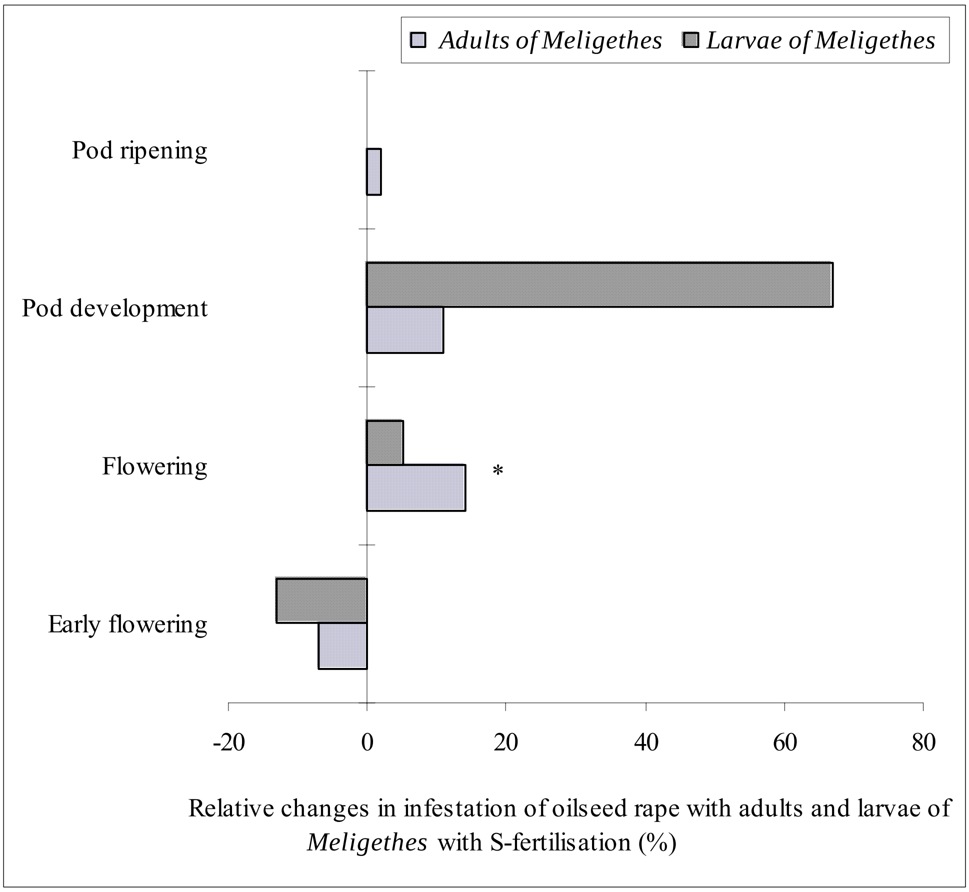


Fig. 3.7: Average changes of the relative infestation level of oilseed rape with adults and larvae of *Meligethes* spp. in relation to S-fertilisation during different growth stages. No larvae were found during pod ripening. *: Denote to a significant difference by U-test at P < 0.05.

3.4 *Influence of S-fertilisation on the number of stem-mining weevils, the rape stem weevil (Ceutorhynchus napi) and the cabbage stem weevil (Ceutorhynchus pallidactylus)*

I *Effect of S- and N-application on the infestation of oilseed rape with adults of Ceutorhynchus napi and Ceutorhynchus pallidactylus*

The first experiment in 2004 showed that S-application increased the infestation with *Ceutorhynchus pallidactylus* relative to control at flowering (BBCH 61, 63, 65, 66) while the infestation level decreased with S-application during pod development (BBCH 75, 76, 78, 81) (Table A.15).

In 2005 another trend was observed. Here the application of S significantly increased the infestation with *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* already very early in crop development (when 9 leaves were build BBCH 19), while S-application decreased infestation with both species at the end of side shoot development (BBCH 29) (Fig. 3.8).

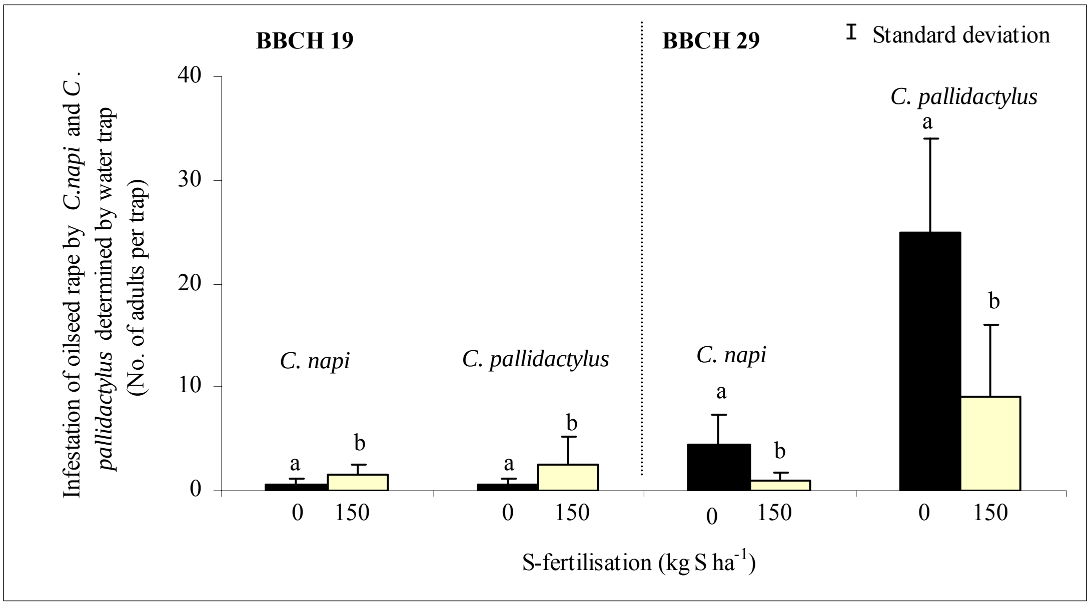


Fig. 3.8: Effect of S-fertilisation on the number of adults of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* (insects collected by yellow water traps in 2005) (different letters denote significant differences between treatments at the 0.05 level by the U-test).

Moreover, S-fertilisation decreased the number of adults of *Ceutorhynchus pallidactylus* during pod development and ripening in 2005. The total number of collected adults of *Ceutorhynchus pallidactylus* was about seven times higher in the S-unfertilised plots compared to S-fertilised plots. This result was found in plants that received a higher N dose (Table A.18).

No significant differences could be observed in the numbers of *Ceutorhynchus pallidactylus* adults in relation to N-application but the infestation level tend to be higher in plots which received the higher N dose of 200 kg ha^{-1} (Table A.18).

The reproduction success of *Ceutorhynchus pallidactylus* was not affect by S-fertilisation.

II Effect of S- and N-application on the infestation with larvae of Ceutorhynchus napi and Ceutorhynchus pallidactylus

The investigation of oviposition preference of females of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* as well as the preference in feeding behaviour of larvae of both species, number and development of larvae were done by stem dissection at different growth stages of oilseed rape.

In the first experiment, the main and second racemes were dissected under a binocular at the beginning of flowering (BBCH 61 and 63). The number of egg deposits (oviposition punctures), egg batches and larvae of both species were counted. The results indicate a strong oviposition preference by females for the S-fertilised plants. Significantly more egg-laying punctures were found in S-fertilised plots at BBCH 61 (Table 3.6). Moreover, the number of laid eggs was also significantly higher in the second racemes of plants which were fertilised with S at BBCH 63 (Fig. 3.9). The number of larvae in the second raceme of plants which were fertilised with S was higher at BBCH 61 and there was also a tendency of a higher infestation of the main raceme of S-fertilised plants (Fig. 3.10).

At the end of flowering (BBCH 67) the number of fully grown larvae was counted just before they migrated to soil for pupation. At this time of investigation more parameters were used to assess plant damage. The following parameters were recorded: length and number of feeding tunnels, infection rate of stems and number of emergence holes. The results indicate again differences with respect to the variety of oilseed rape. The length of feeding tubes was significantly higher in S-fertilised plants of Bristol, while no significant differences were found for Lipton (Table 3.7) and the percentage of infested stems was significantly higher in the main and second racemes of S-fertilised plants of the variety Bristol (Fig. 3.11) (A).

The infection severity with *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* larvae were determined by counting the number of feeding tubes per plant. The number of feeding tubes (lesion areas) in the main racemes was significantly higher in S-fertilised plants while no significant differences were found in the second raceme (Table 3.7). It can be concluded from the results that S-fertilisation enhanced and increased the infection severity with larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* at flowering.

Table 3.6: Influence of S-application on the infestation of oilseed rape with larvae of *Ceutorhynchus napi* and *C. pallidactylus* at the beginning of flowering (BBCH 61).

Parameters	**S- fertilisation (kg ha^{-1})**	
	0	**150**
No. of eggs in the main raceme per one plant	1.83 a	1.95 a
No. of larvae in the main raceme per one plant	0.28 a	0.68 b
Oviposition punctures in the main raceme per one plant	6.00 a	6.08 a
No. of eggs in the second raceme per one plant	9.55 a	8.83 a
No. of larvae in the second raceme per one plant	0.98 a	0.75 a
Oviposition punctures in the second raceme per one plant	16.8 a	16.8 a
No. of eggs per one plant	11.4 a	10.8 a
No. of larvae per one plant	1.28 a	1.49 a
Oviposition punctures per one plant	22.8 a	23.1 a

Mean values followed by the different letters indicate significant differences by U-test at 0.05 levels. n: 40 for all treatments.

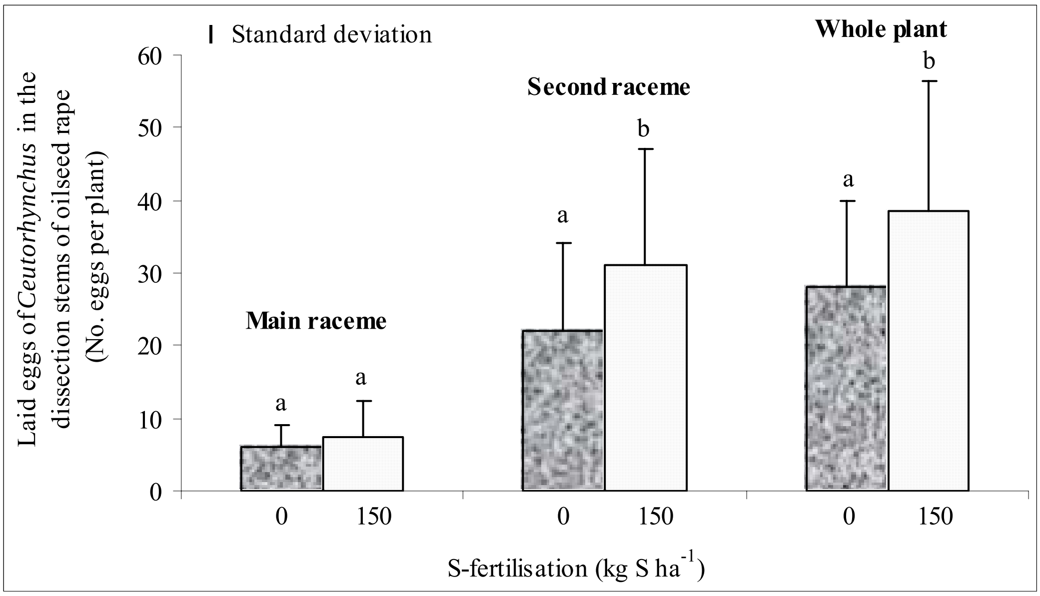


Fig. 3.9: Influence of S-fertilisation on the number of *Ceutorhynchus* laid eggs at the beginning of flowering (BBCH 63) in the main raceme, second raceme and whole plant (main and second racemes) in 2004 (different letters denote significant differences between S-fertilisation treatments at the 0.01 level by the U-test).

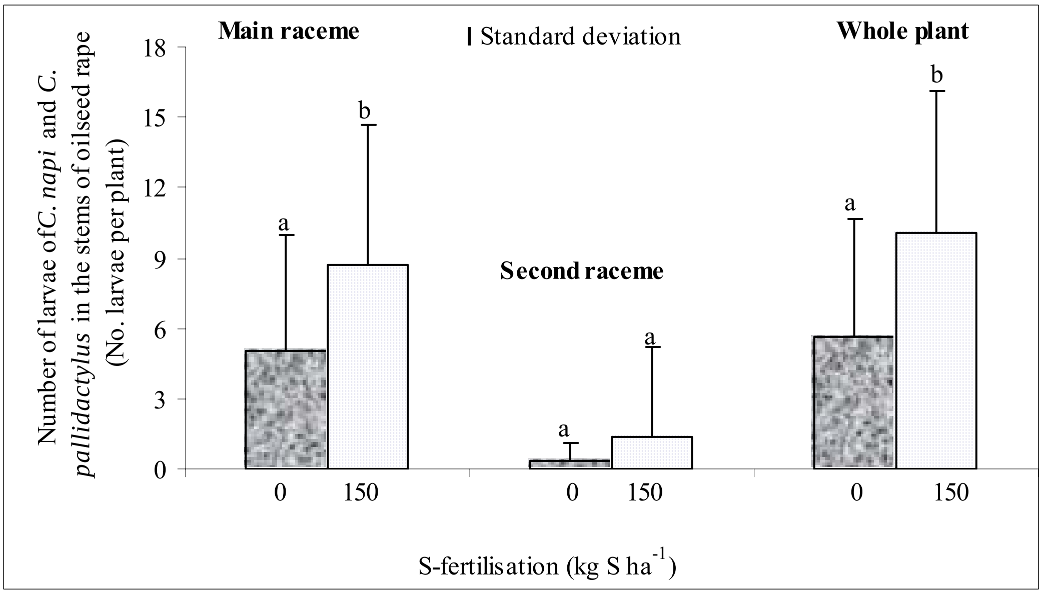


Fig. 3.10: Effect of S-application on the number of larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* at flowering (BBCH 63) in the main raceme, second raceme and whole plant (main and second racemes) in 2004 (different letters denote significant differences between S-fertilisation treatments at the 0.01 level by the U-test).

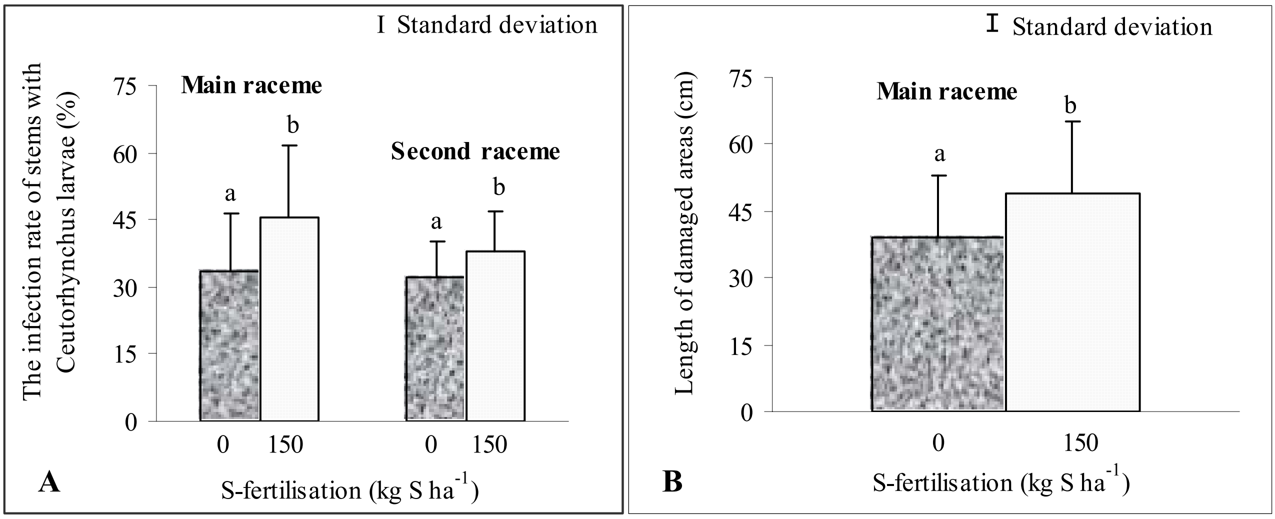


Fig. 3.11: Effect of S-application on the percentage of infected stems of oilseed rape (Bristol var.) with larvae (A) and length of damaged areas of the main raceme which were infected by larvae (B) in relation to S-fertilisation at the end of flowering (BBCH 67) in 2004 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

Table 3.7: Influence of S-application on the infestation of oilseed rape with larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* at flowering (BBCH 67) in 2004.

Parameters	**S- fertilisation (kg ha^{-1})**	
	0	**150**
% Infection of head raceme	41.7 a	49.1 b
length of feeding tubes in the main raceme	50.8 a	54.8 a
No. of feeding tubes per plant in the main raceme	1.73 a	2.00 a
No. of larvae in the main raceme per plant	24.9 a	23.9 a
No. of larvae in the main raceme per m^2	1494 a	1434 a
No. of emergence holes per plant in main raceme	4.98 a	4.18 b
No. of feeding tubes per plant in second raceme	1.11 a	1.22 a
No. of larvae in the second raceme per plant	5.77 a	5.17 a
No. of larvae in the second raceme per m^2	346 a	310 a
% Infection of second raceme	34.1 a	37.6 b
Length of feeding tubes per plant in second raceme	27.1 a	30.1 b

Mean values followed by the different letters indicate significant differences by U-test at 0.05 levels. n: 80 for all treatments.

This study showed that S-fertilisation positively affected the oviposition behaviour of female of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* (Table 3.6). Also the number of first and second instars of both species (BBCH 63) significantly increased. The feeding damage caused by larvae of both species increased with S-fertilisation (Table 3.7). But when looking at the results for *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* separately it became obvious that the number of larvae of *Ceutorhynchus napi* was lower in S-fertilised plants, while the larvae of *Ceutorhynchus pallidactylus* did not vary in relation to S-fertilisation at end of flowering (BBCH 67) (Table 3.8).

Table 3.8: Influence of S-application on the number of larvae of *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* collected by plant stem dissection (BBCH 67) (data from 2004).

Species	**Number of larvae**	
	0 kg S ha^{-1}	**150 kg S ha^{-1}**
Ceutorhynchus napi	10.7 a	6.00 b
Ceutorhynchus pallidactylus	44.2 a	44.7 a

Mean values followed by different letters indicate significant differences between the S-treatments by U-test at 0.01 level. N: 80.

The infection rate of stems, the length of feeding tubes, the number of larvae and the number of emergence holes were determined to show the extend of infestation in the second experimental year. In 2005 a significant influence of the S-fertilisation was observed. S-fertilisation significantly decreased the level of infestation with *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* (Table A.19). The length of feeding tubes (areas damaged by larvae) (Fig. 3.12), the infestation rate of the stems (Fig. 3.13), the numbers of exit holes and larvae (Table A.19), and the number of feeding tubes which is an indicator for the infestation severity decreased with S-fertilisation (Table A.19).

Nitrogen application on the other hand seemed to increase the infestation by larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus*. The length of feeding tubes and the percentage of infested stems were significantly higher in plants which were fertilised with the higher dose of N (200 kg N ha^{-1}) (Fig. 3.12 and Fig. 3.13).

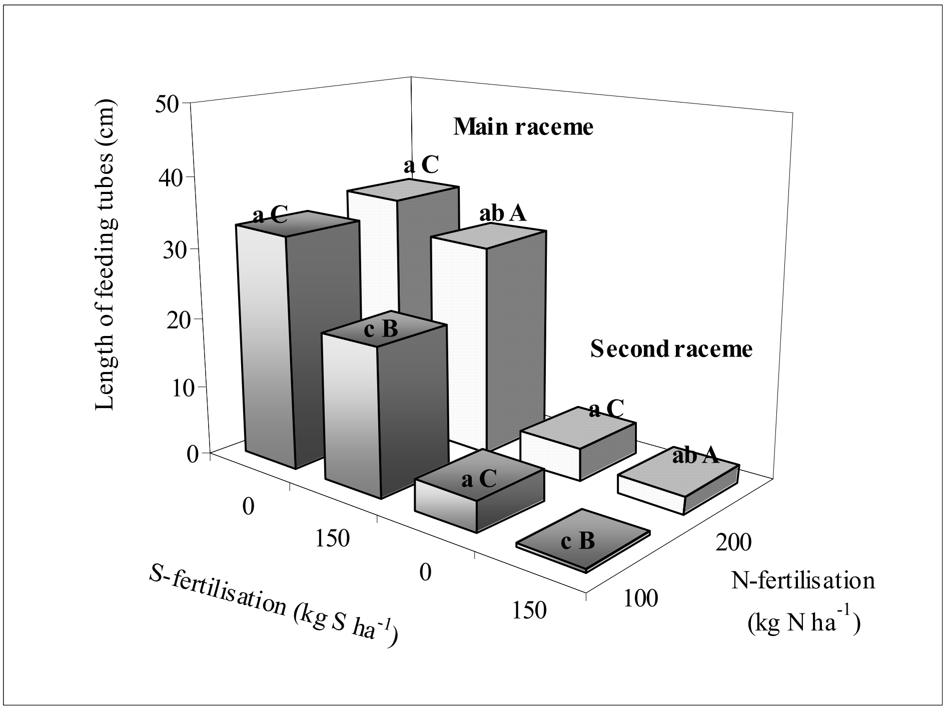


Fig. 3.12: Effect of S- and N-supply on the length of damaged areas caused by larvae of *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* in the stems of oilseed rape (var. Lion) at pod development (BBCH 76) in 2005 (different lowercase letters denote to significant differences between S-treatments and different uppercase letters denote to significant differences between N-treatments at the 0.05 level by U-test).

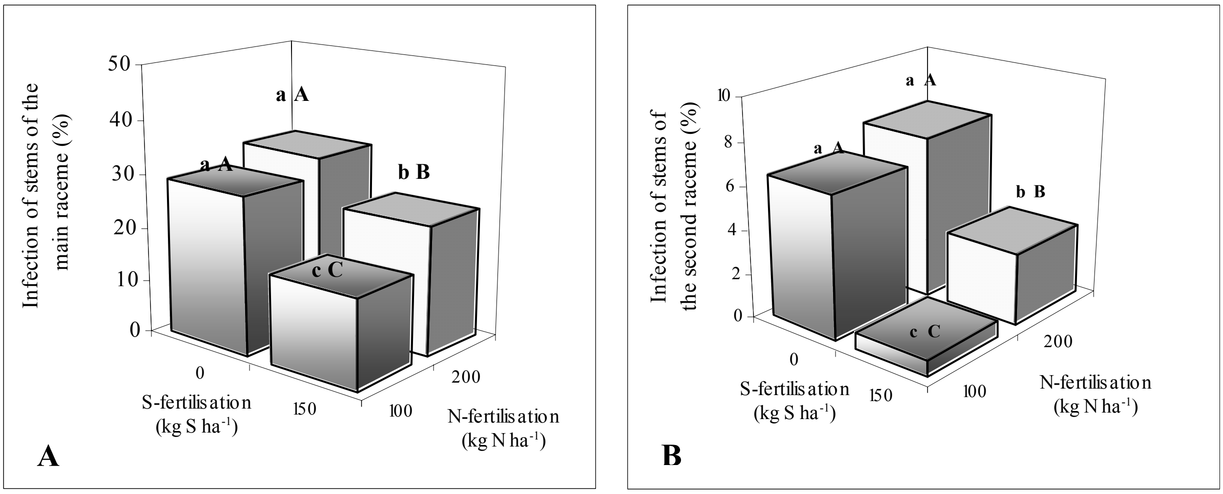


Fig. 3.13: Effect of S- and N-application on the percentage of infested stems (main racemes (A) and second racemes (B) of oilseed rape) with larvae of *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* at pod development (BBCH 76) in 2005 (different lowercase letters denote to significant differences between S-treatment and different uppercase letters denote to significant differences between N-treatment at the 0.05 level by U-test).

Plants are susceptible to *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* in early spring, when female adults appear from hibernation and invade into oilseed rape plants to deposit eggs, and during stem elongation, when larvae bore inside the stems to feed on them. The results from this study show that S-application increased the incidence of *Ceutorhynchus pallidactylus* adults during flowering. The higher number of female adults resulted in higher numbers of oviposition punctures and egg butches (Table 3.6), enhanced the number of first and second instars at BBCH 63 and increased the damage caused by third instars or full grown larvae at BBCH 67. But later in the growing season at pod development a significant lower damage was observed in plots which received an S-application.

3.5 *Influence of S-fertilisation on the number of the cabbage seed weevil* (*Ceutorhynchus obstrictus*)

I *Effect of S- and N-application on the infestation of oilseed rape with adults of Ceutorhynchus obstrictus*

The results of the different traps indicate that S-fertilisation increased the occurrence of *Ceutorhynchus obstrictus*, especially at early flowering and full ripening (Table A.20). A significantly higher number of adults of *Ceutorhynchus obstrictus* was collected by sweep net at early flowering (BBCH 61) and when 60% of the flowers of the main raceme were open (BBCH 66) (Fig. 3.14) (A). The same result was observed by emergence traps at pod ripening (BBCH 81). A significantly higher number of adults from the new generation was caught in S-fertilised plots (Fig. 3.14) (B).

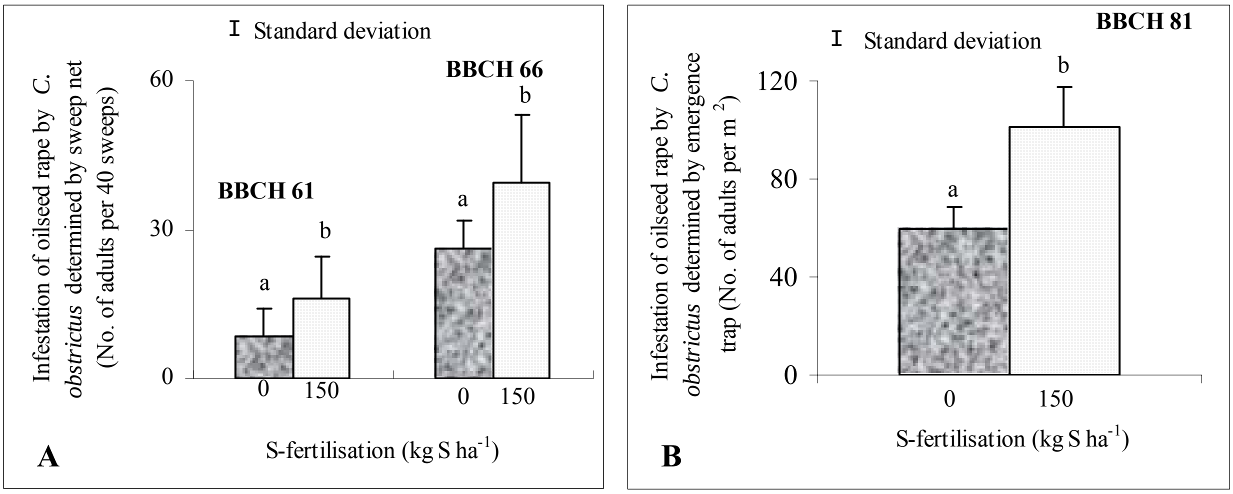


Fig. 3.14: Infestation with adults of *Ceutorhynchus obstrictus* in relation to the S-nutritional status of oilseed rape at different growth stages in 2004 monitored by sweep net (A) and emergence traps (B) (different letters denote to significant differences between S-treatments at the 0.05 level by the U-test).

The results of the second year emphasise the importance of the S- and N-fertilisation on the susceptibility of oilseed rape to adults of *Ceutorhynchus obstrictus* during different growth stages. A significantly higher number of adults of *Ceutorhynchus obstrictus* was collected in plots which were fertilised with S by using suction trap (Fig. 3.15) and sweep net (Fig. 3.16) at different growth stages. Despite of the fact that a higher number of adults was collected in S-fertilised plots during different growth stages a significantly lower number of adults of the new generation of *Ceutorhynchus obstrictus* were collected by emergence traps from the S-fertilised plots at BBCH 83 (Table A.22). The beating tray delivered contradictory results compared to the sweep net and suction trap (Fig. 3.17) (A).

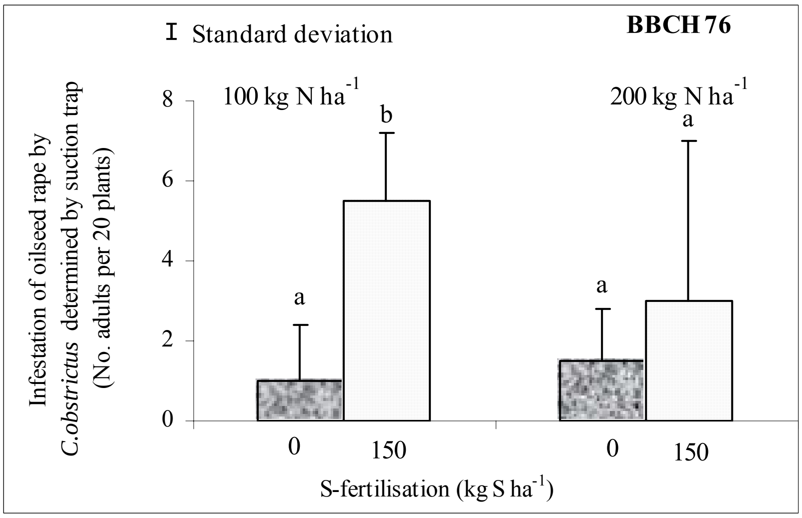


Fig. 3.15: Infestation of oilseed rape (var. Lion) with adults of *Ceutorhynchus obstrictus* in relation to S-nutrition at main pod development in 2005 (sig: denote to significant differences between treatments at the 0.007 level by the U-test).

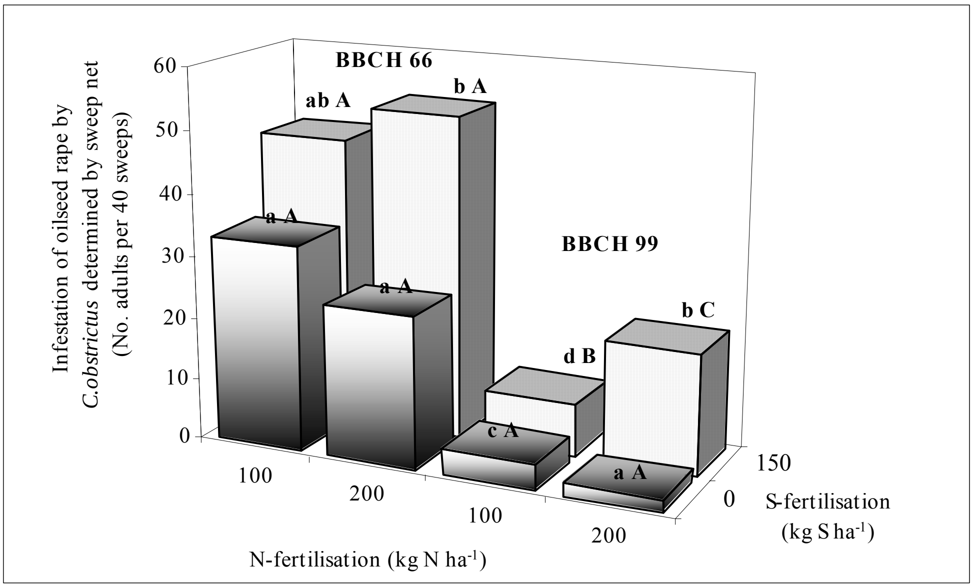


Fig. 3.16: Influence of S- and N-fertilisation on the infestation of oilseed rape (var. Lion) with adults of *Ceutorhynchus obstrictus* at different growth stages in 2005 (different lowercase letters denote to significant differences with S-fertilisation and different uppercase letters denote to significant differences with N-fertilisation at the 0.05 level by the U-test).

Adults of *Ceutorhynchus obstrictus* also seem to respond to N-nutrition in that way that the number of adults was significantly higher on plants that received the higher dose of 200 kg N ha^{-1} at different growth stages (BBCH 75 and 99) (Fig. 3.16, 3.17 B). The infestation level also increased with N-fertilisation during pod development and ripening (BBCH 72, 76, 78 and 83) as shown in the appendix (Table A.23).

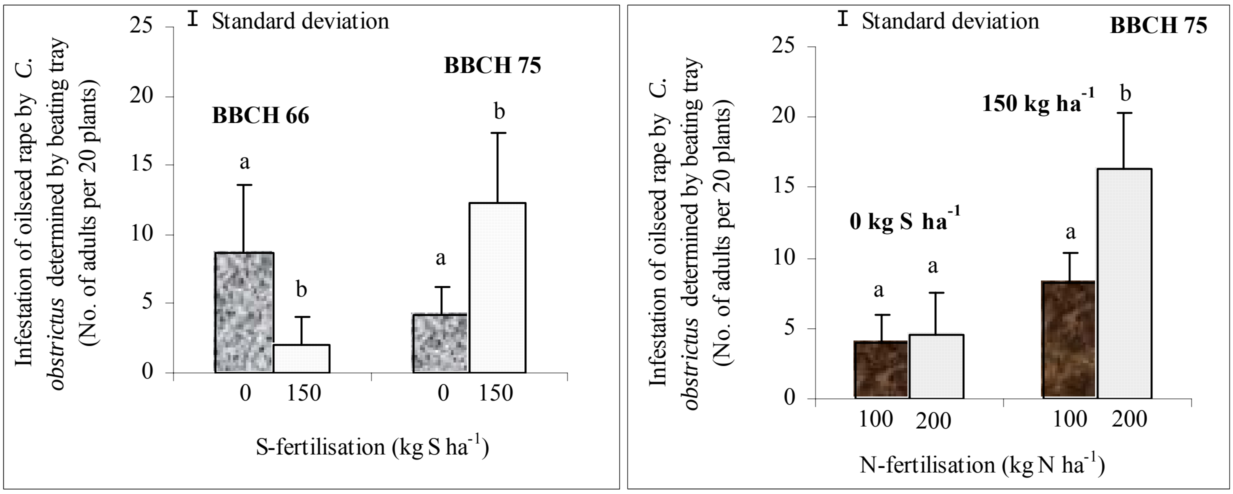


Fig. 3.17: Infestation of oilseed rape (var. Lion) by *Ceutorhynchus obstrictus* in relation to S-fertilisation (A) and N-application (B) monitored by beating tray during different growth stages in 2005 (different letters denote to significant differences between treatments at the 0.01 level by the U-test).

Reproduction success of *Ceutorhynchus obstrictus* was increased about 87% by S-fertilisation (from 0.39 to 0.73).

II Effect of S- and N-application on the infestation of oilseed rape with larvae of Ceutorhynchus obstrictus

Larvae of the *Ceutorhynchus obstrictus* were collected by funnel traps from pod and seed development until harvest. Moreover the infestation level was determined two times by pod dissection when 30% and 50% of the pods reached their final size (BBCH 73 and 75).

In 2004 no significant differences were observed in the infestation rate during different growth stages in relation to S-application but S-fertilisation increased the occurrence of *Ceutorhynchus obstrictus* larvae in the main and second raceme of plants when 30% of pods reached their final size (BBCH 73). A significantly higher infestation rate with larvae of *Ceutorhynchus obstrictus* was observed when focussing on the data of oilseed rape variety Bristol (Fig. 3.18).

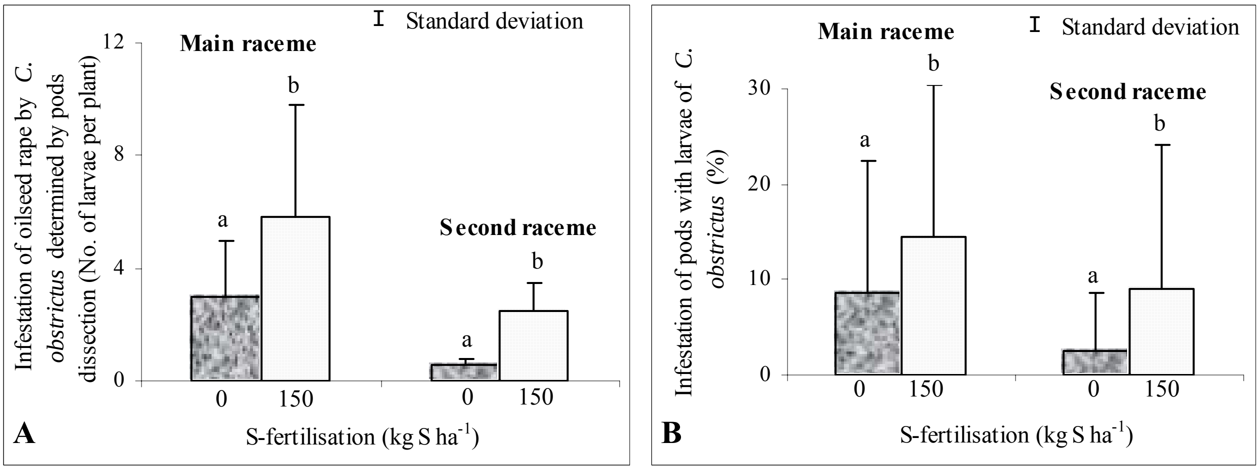


Fig. 3.18: Effect of S-fertilisation on the infestation of oilseed rape (var. Bristol) with larvae of *Ceutorhynchus obstrictus* (A) and on the percentage of infested pods (B) at pod development (BBCH 73) in 2004 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

In 2005 the influence of S-fertilisation on infestation with larvae of *Ceutorhynchus obstrictus* differed in relation to the growth stage of oilseed rape. At full pod development (BBCH 75, 76) infestation decreased with S-fertilisation while later in pod ripening at BBCH 81 the opposite was observed (Fig. 3.19) (A).

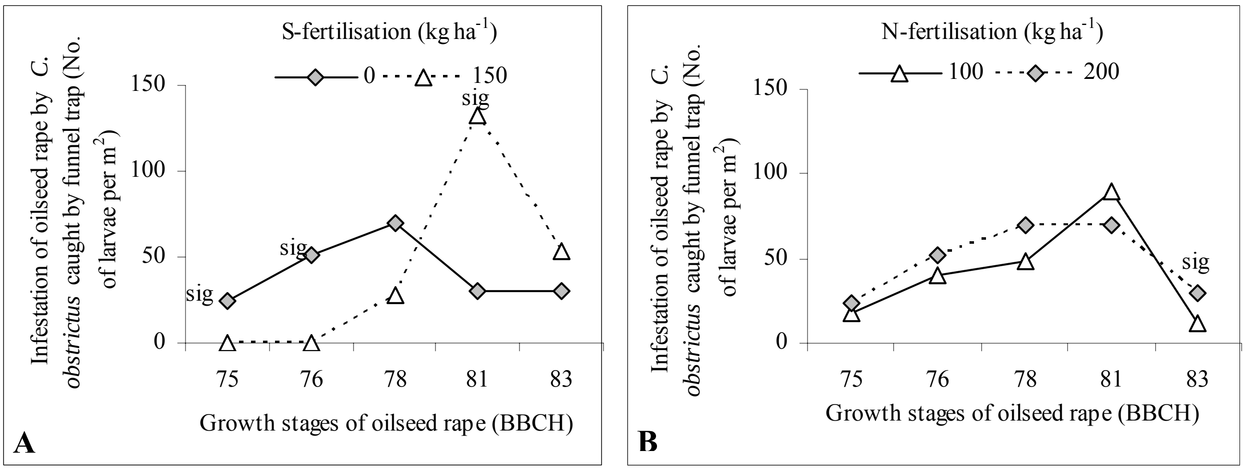


Fig. 3.19: Infestation of oilseed rape (var. Lion) with larvae of *Ceutorhynchus obstrictus* in relation to S-application (A) and N-application (B) (sig. denote to significant differences between treatments at the 0.05 level by the U-test) (season 2004/2005).

It was also observed that the infestation with larvae of *Ceutorhynchus obstrictus* tends to be a little bit higher with a higher N-fertiliser level. Significantly higher numbers of larvae were collected from plants which were fertilised with 200 kg N ha^{-1} compared to plots that received 100 kg N ha^{-1} at BBCH 83 as Fig. 3.19 (B) shows.

A pod dissection was conducted at BBCH 75 and the results coincided with the results which were obtained with funnel traps. S-fertilisation decreased the larval infestation of the main raceme (Fig. 3.20) (A) and also the infection rate of pods with *Ceutorhynchus obstrictus* larvae (Fig. 3.20) (B).

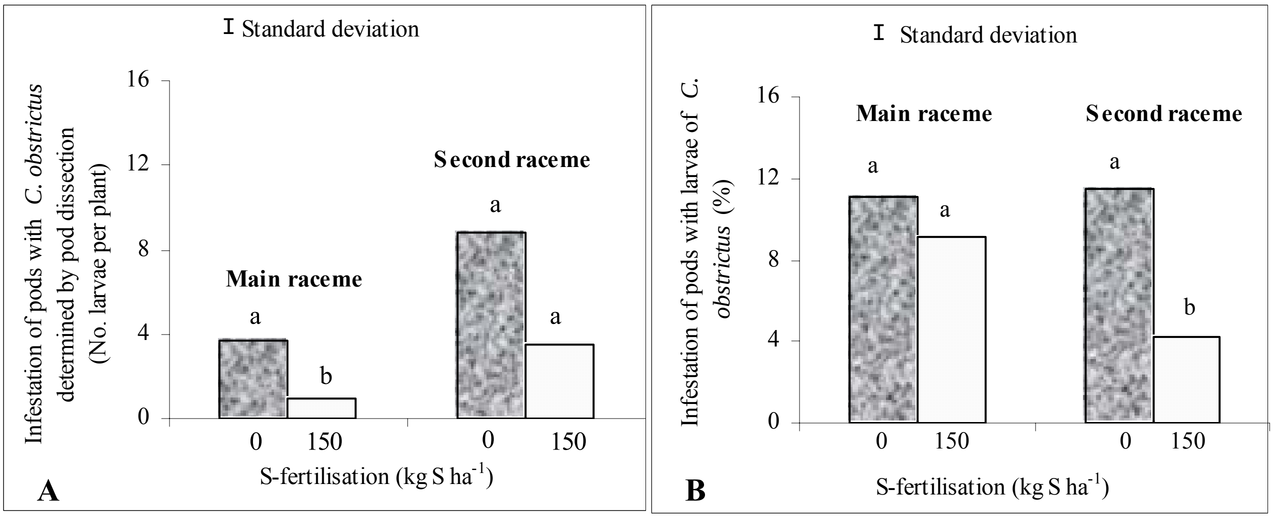


Fig. 3.20: Influence of S-application on the infestation of oilseed rape (var. Lion) with larvae of *Ceutorhynchus obstrictus* (A) and on the percentage of infested pods (B) at full pod development (BBCH 75) in 2005 (different letters denote significant differences between treatments at the 0.05 level by the U-test).

The results of most trapping methods indicate that S-fertilisation increased the incidence of *Ceutorhynchus obstrictus* adults during different growth stages (Table A.20, A.21, and A.22). Significantly higher infestation rates with adults were found at early flowering (BBCH 61, 62) and at full flowering (BBCH 64) and an increase with S-fertilisation was also observed during pod development (BBCH 71 to 78). Furthermore the new generation of adults was positively affected by S-fertilisation (BBCH 97 and 99). S-fertilisation increased the infestation rate with larvae at BBCH 73, 78, 81, and 83 (Table A.24). Adults and larvae of *Ceutorhynchus obstrictus* also respond positively to higher N levels (Table A.23, A.24).

This study indicated that different species of *Ceutorhynchus* genus (*Cleutorhynchus pallidactylus*, *Ceutorhynchus obstrictus*, and *Ceutorhynchus napi*) were positively affected by S-fertilisation during early spring, flowering, and pod ripening (Fig. 3.21).

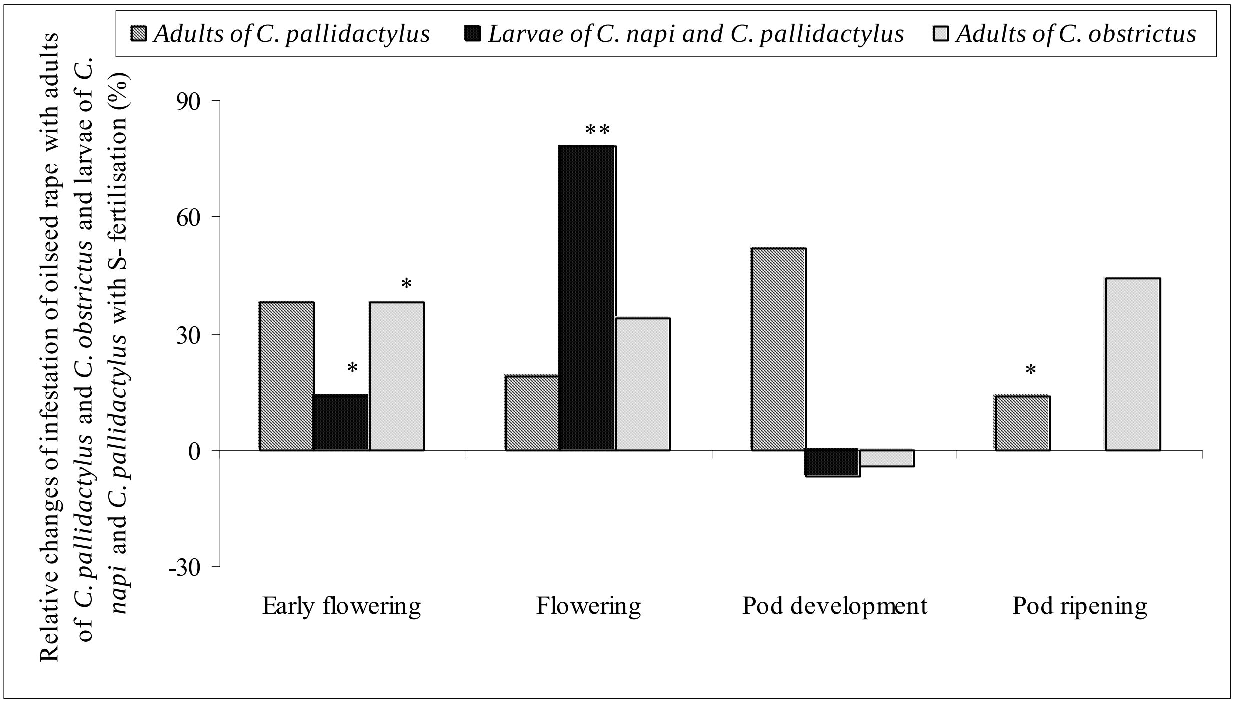


Fig. 3.21: Relative changes in infestation of oilseed rape with adults of *Ceutorhynchus obstrictus* and *Ceutorhynchus pallidactylus* as well as larvae of *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* in relation to S-fertilisation in 2004 (no larvae were found at pod ripening stage) (*: Denote to a significant differences by U-test at $P < 0.05$, * *: Denote to a significant differences by U-test at $P < 0.01$).

3.6 Influence of S-fertilisation on the number of Brassica pod midge (Dasineura brassicae)

I Effect of S- and N-application on the infestation of oilseed rape with adults of Dasineura brassicae

Adults of *Dasineura brassicae* were collected from flowering until harvest by different methods. In the first experimental year in 2004 a positive influence of S-application on the occurrence of adults of *Dasineura brassicae* was observed (Fig. 3.22).

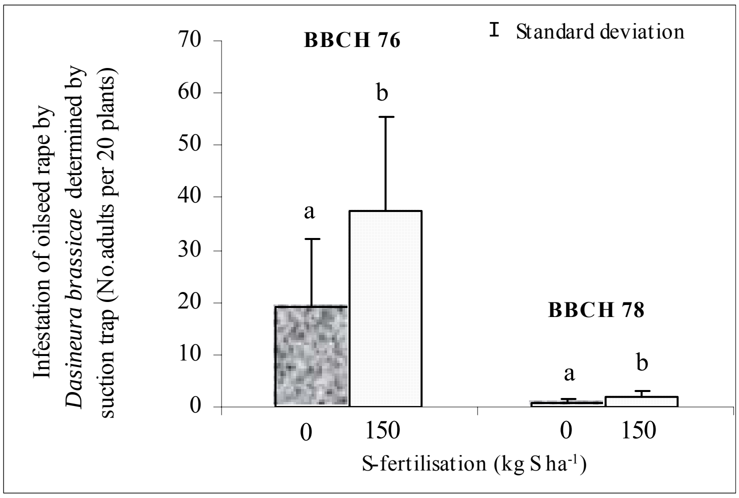


Fig. 3.22: Influence of S-fertilisation on the infestation of oilseed rape (var. Bristol) with adults of *Dasineura brassicae* collected with suction trap at pod development in 2004 (different letters denote to significant differences between S-treatments at the 0.05 level by the U-test).

The results from 2005 are consistent with the results from 2004. The relative infestation rate with adults of *Dasineura brassicae* increased significantly with S-fertilisation when 60% of the flowers were open (BBCH 66) and when the second generation of adults was collected by sweep net and suction trap (BBCH 83) (see appendix Table A.27). Only one exception was found when the first generation of adults was collected at full pod development (BBCH 75, 76) by sweep net and suction trap in 2005. This result indicated that S-fertilisation decreased the number of adults of the first generation and delayed their peak occurrence at BBCH 77 (Fig. 3.23). Furthermore, the total numbers of adults of the second generation which were captured by emergence traps over the whole ripening stage was significantly higher in S-fertilised plots (Fig. 3.24). This decrease in number could be due to influence of landscape structure at this time.

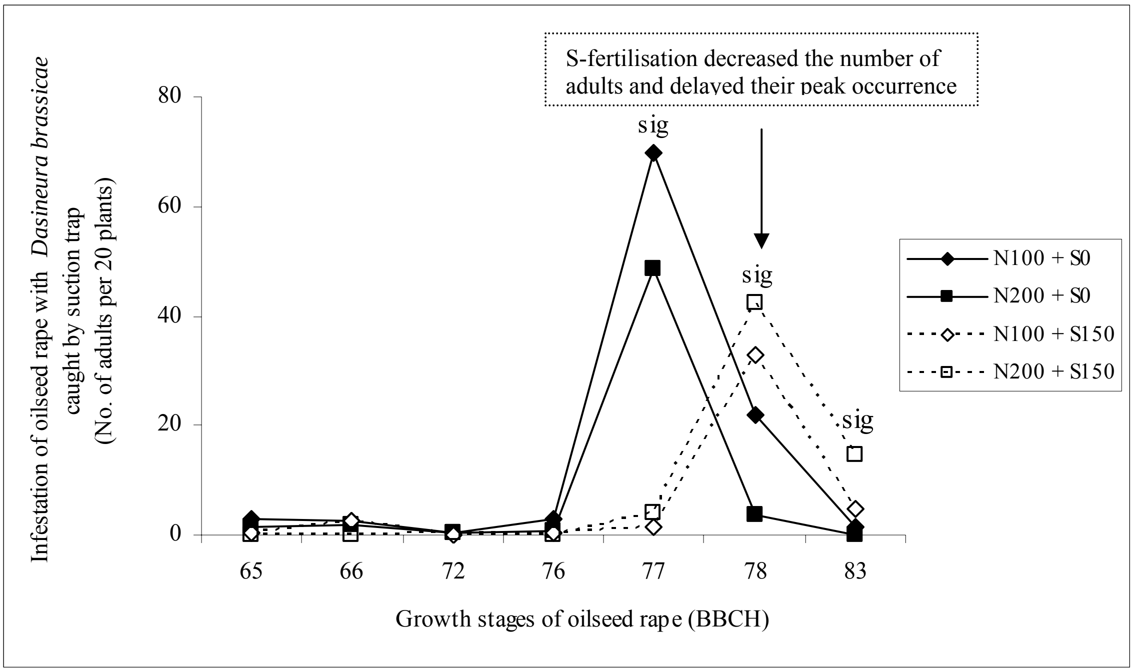


Fig. 3.23: Infestation of oilseed rape (var. Lion) with *Dasineura brassicae* at different growth stages in relation to S-fertilisation in 2005 (sig. denote to significant differences between treatments at the 0.05 level by the U-test) (S0 plots without S-application, S150 plots which received 150 kg S ha^{-1}, N100 plants which received 100 kg N ha^{-1} while N 200 plants that fertilised with 200 kg N ha^{-1}).

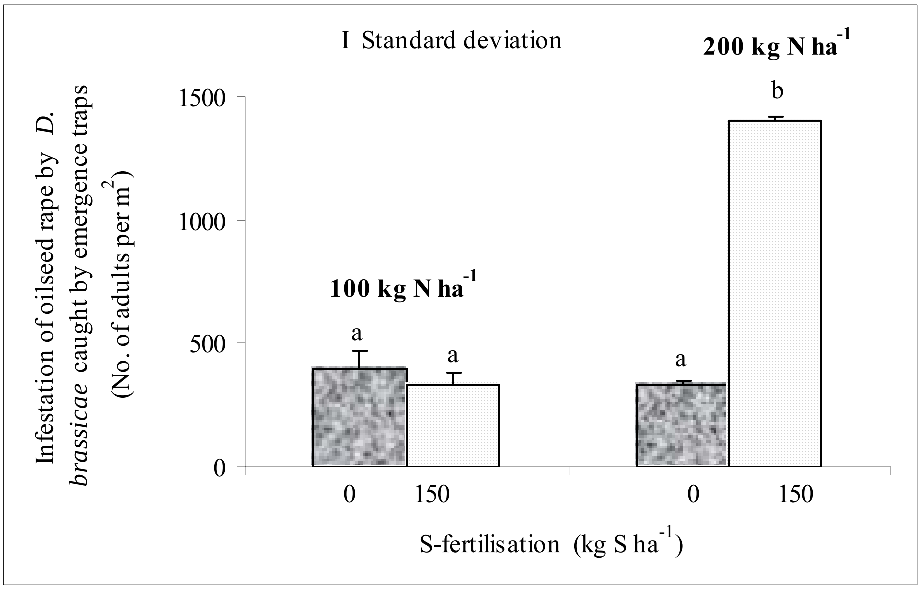
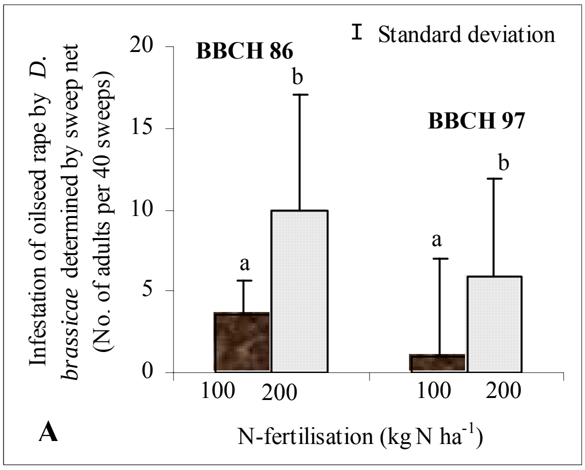


Fig. 3.24: Effect of S-fertilisation on the number of *Dasineura brassicae* adults collected by emergence traps during the whole-season 2004/2005 in relation to N-supply (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

N-fertilisation increased the occurrence of *Dasineura brassicae* at most growth stages of oilseed rape (Table A.28). Significantly more adults were collected from plants that received the higher dose of N-application (200 kg N ha^{-1}) at BBCH 83, 86 and 97 (Fig. 3.23, 3.25).



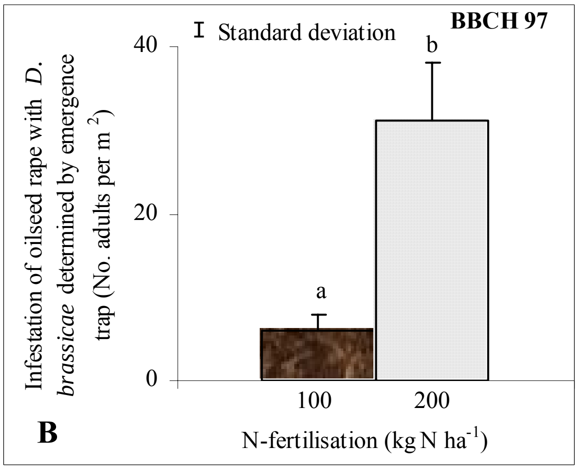


Fig. 3.25: Influence of N-fertilisation on the infestation of oilseed rape (var. Lion) with adults of *Dasineura brassicae* caught by sweep net (A) and emergence traps (B) at different growth stages in 2005 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

In this study the reproduction success of this pest was calculated by counting number of new generation of adults and number of full grown-larvae per m^2. The result showed that S-supply decreased reproduction success of *Dasineura brassicae* from 0.13 in S-unfertilised plants to 0.11 in S-fertilised.

II Effect of S- and N-application on the infestation with larvae of Dasineura brassicae

Larvae of *Dasineura brassicae* were collected from end of flowering until harvest by sweep net and funnel traps. Additionally, larvae were collected from dissected pods and the level of pods which were infested with larvae was determined. A significantly higher number of larvae of the second generation were collected from S-fertilised plots by funnel traps in 2004. An increase of infestation with S-fertilisation was observed during the whole vegetation period of oilseed rape (Fig. 3.26).

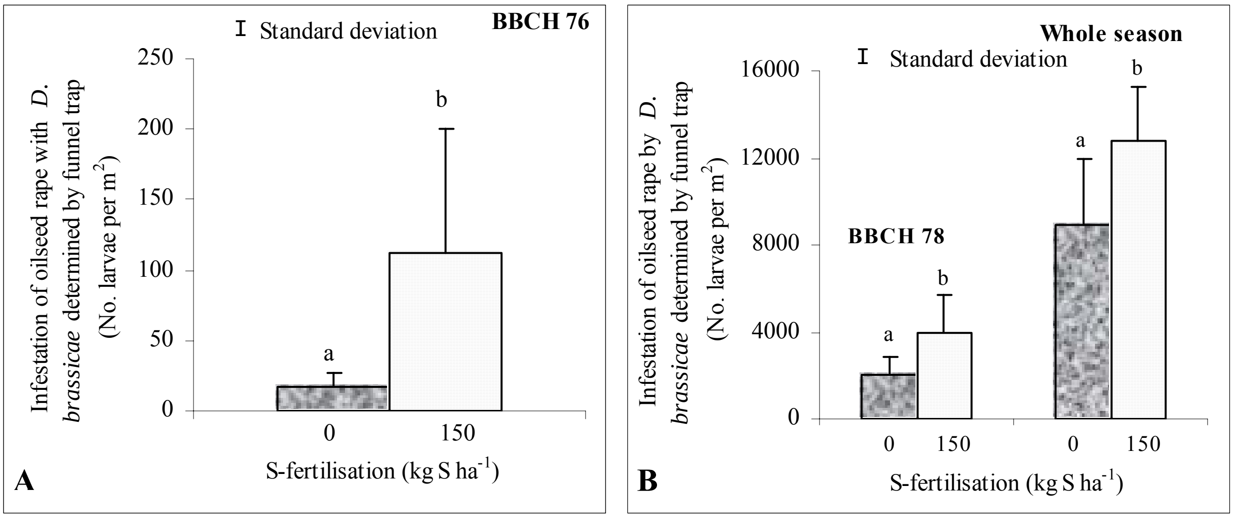


Fig. 3.26: Effect of S-fertilisation on the infestation of oilseed rape with larvae of *Dasineura brassicae* caught by funnel traps at full pod development (A) and in whole 2003/2004 season (B) (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

Larvae of the first generation were collected at BBCH 73 from the main and second racemes by pod dissection. The percentage of infested pods was determined as well as the level of infestation by larvae of *Dasineura brassicae* and both parameters were significantly higher in S-fertilised plots (Fig. 3.27).

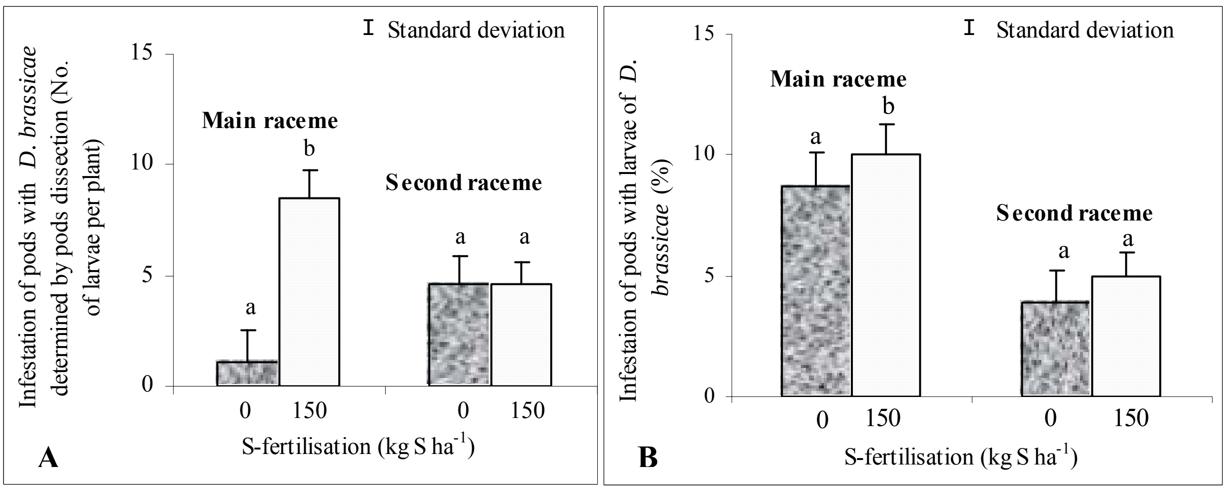


Fig. 3.27: Effect of S-fertilisation on the infestation of oilseed rape (var. Lipton) with larvae of *Dasineura brassicae* (A) and on the percentage of infestation (B) at pod development (BBCH 73) in 2004 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

Also in 2005, a positive influence of S-application on the occurrence of *Dasineura brassicae* larvae was observed during different growth stages. For example significantly higher numbers of larvae were collected by funnel traps from S-fertilised plants at the end of flowering (BBCH 67), and when the second-generation of larvae were collected at BBCH 81 (Fig. 3.28).

Also, the number of larvae from the first-generation was determined and the infection rate of pods when 50% of pods reached their final size (BBCH 75). In 2005 significantly higher numbers of larvae in the main raceme were found in S-unfertilised plots (Fig. 3.29) (A) and also the infection rate of pods was higher when no S-fertilisation was applied (Fig. 3.29) (B).

N-fertilisation seem to increase the level of infestation with *Dasineura brassicae* because a much higher level of infestation was observed when 200 kg N ha^{-1} was fertilised in comparison to the lower dose of only 100 kg N ha^{-1}.

Adults of *Dasineura brassicae* as specialists of oilseed rape locate their host plants by compounds which are related to the S-nutritional status (see introduction) like GSLs. Therefore it was most likely that S-fertilisation had an influence on the infestation level. S-application significantly increased the number of adults during the full flowering period (BBCH 66). Adults of the first and second generation were significantly more attracted by plots which were fertilised with S (Fig. 3.22). Moreover also a higher N-fertilisation caused a significantly higher infestation with *Dasineura brassicae* (adults and larvae).

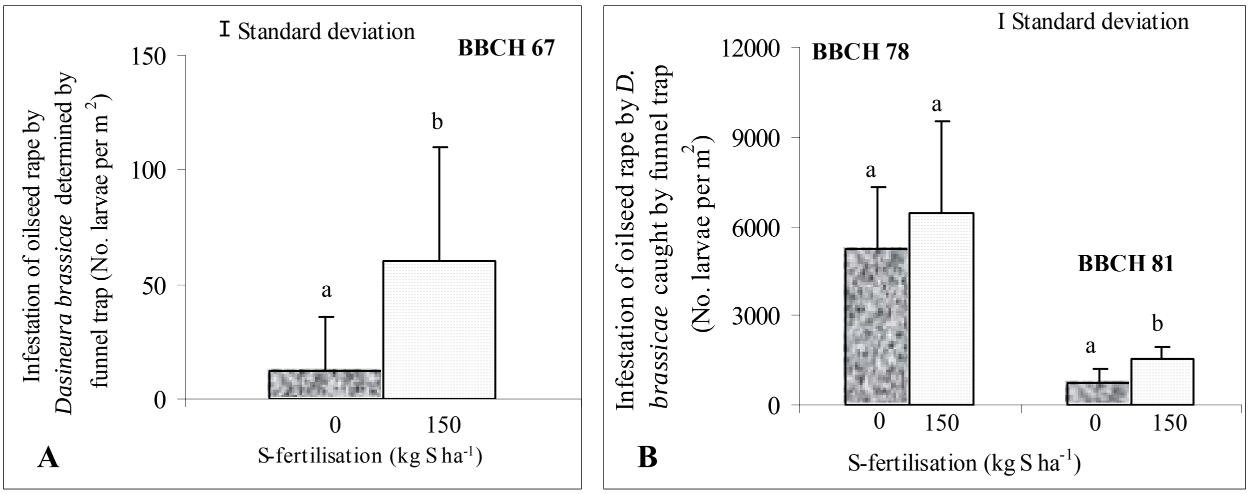


Fig. 3.28: Influence of S-application on the infestation of oilseed rape with larvae of *Dasineura brassicae* (larvae caught by funnel traps at end of flowering (A) and at pod development (B) in 2005) (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

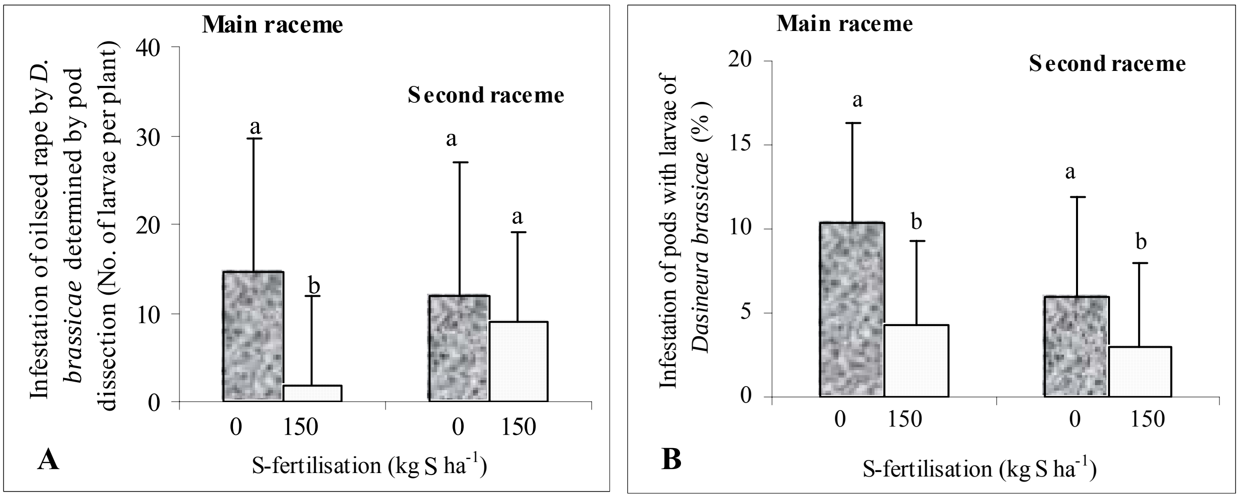


Fig. 3.29: Effect of S-fertilisation on the number of *Dasineura brassicae* larvae (A) and the percentage of infested pods (B) at full pod development (BBCH 75) in 2005 (different letters denote to significant differences between treatments at the 0.01 level by the U-test).

In conclusion this study showed that the infestation of oilseed rape plants by adults and larvae of *Dasineura brassicae* increased with S-supply during all main growth stages of oilseed rape (Fig. 3.30).

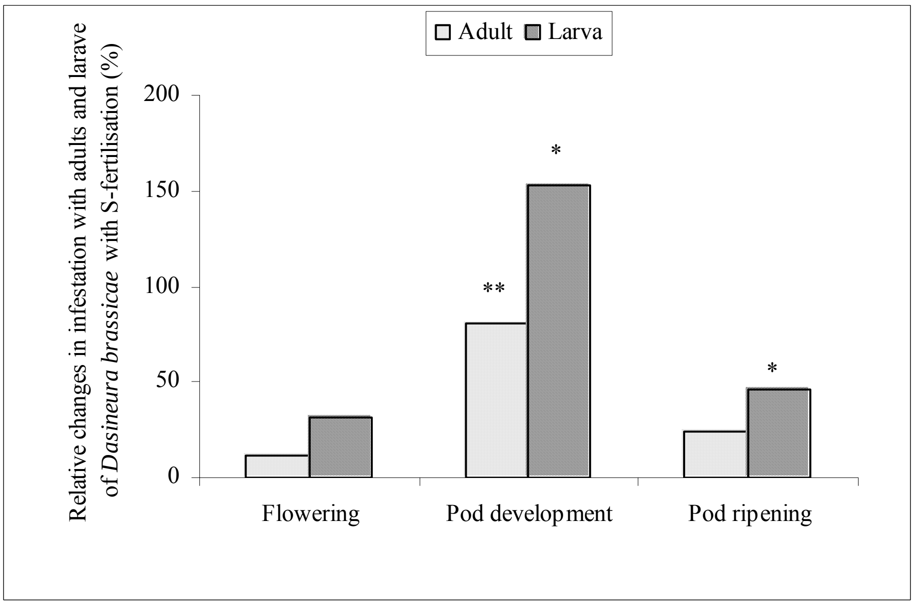


Fig. 3.30: Relative changes in infestation with adults and larvae of *Dasineura brassicae* with S-fertilisation at main growth stages of oilseed rape in 2004 (*: Denote to a significant differences by U-test at P < 0.05, **: Denote to a significant differences by U-test at P < 0.01).

3.7 *Influence of S-fertilisation on the number of root flies (Delia radicum, Delia platura and Delia florilega)*

I *Effect of S- and N-fertilisation on the infestation of oilseed rape with adults of Delia radicum*

Adults of *Delia radicum* showed a positive response to S-fertilisation during most growth stages of oilseed rape (Table A.30). Significantly higher numbers of adults were collected from S-fertilised plots when all pods reached their final size (BBCH 79) (Fig. 3.31). Also adults of the new generation which were collected by emergence traps appeared in higher numbers in S-fertilised plots (Fig. 3.32).

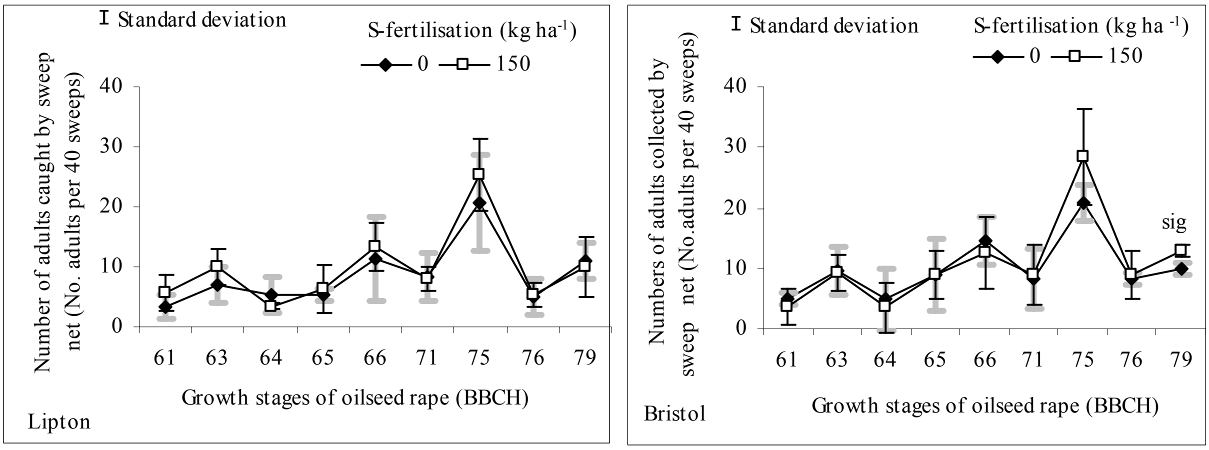


Fig. 3.31: Effect of S-fertilisation on the number of *Delia radicum* collected by sweeps net during different growth stages of oilseed rape in 2004 (sig. denote to significant differences between treatments at the 0.05 level by the U-test).

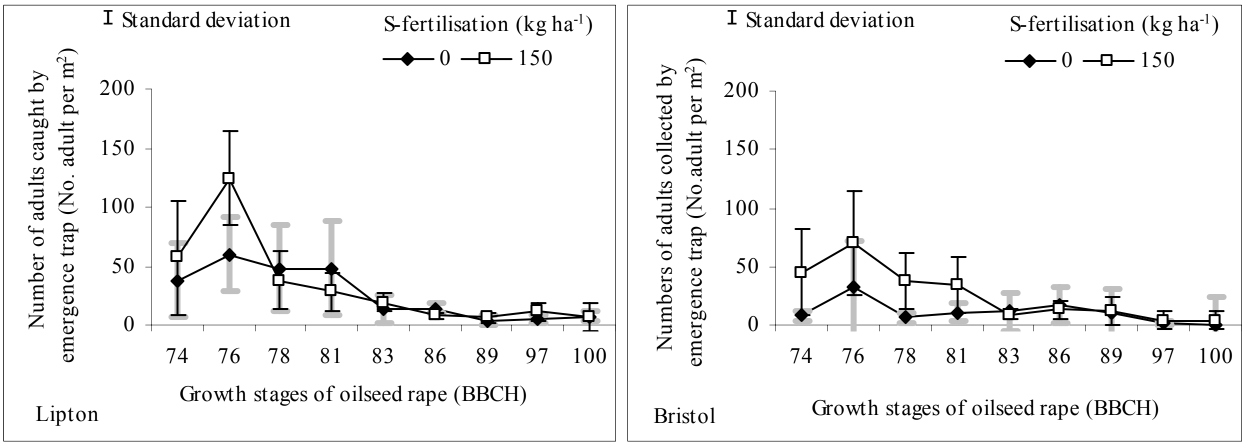


Fig. 3.32: Numbers of adults of *Delia radicum* which were collected by emergence traps during different growth stages of oilseed rape relative to S-fertilisation in 2004.

The opposite trend to 2004 was observed in 2005. In 2005 a significantly higher number of adults was collected from S-unfertilised plots. This result was observed early in plant development when the flower buds raised above the youngest leaves (BBCH 53), but also later at full flowering (BBCH 64), at full pod development (BBCH 75), at the beginning of pod ripening (BBCH 71, 83) and at the harvest of the crops (BBCH 99) (Fig. 3.33).

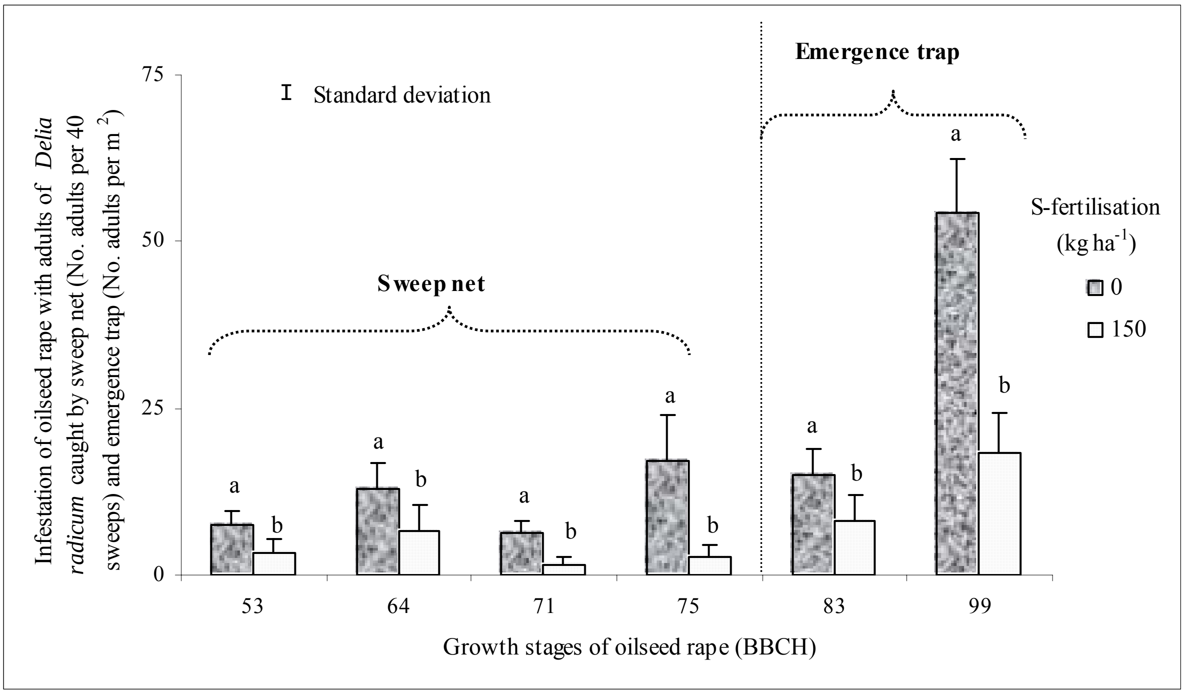


Fig. 3.33: Effect of S-fertilisation on the number of adults of *Delia radicum* which were collected during different growth stages of oilseed rape (var. Lion) by different traps in 2005 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

N-fertilisation had a significantly positive effect on the incidence of *Delia radicum* during different growth stages and monitored with different trapping methods (Table A.30). A significantly higher number of adults was captured from plants that received the higher dose of N (200 kg N ha^{-1}) at pod development and during ripening (BBCH 76, 81) (Fig. 3.34).

The percentage of roots which were infected with larvae of root flies was determined when 4-5 leaves were unfolded (BBCH 14-15). A significantly higher infection rate was found in S-unfertilised plots (Fig. 3.35) (A) and larvae preferred to feed on plants that received a higher dose of N (200 kg N ha^{-1}) (Fig. 3.35) (B).

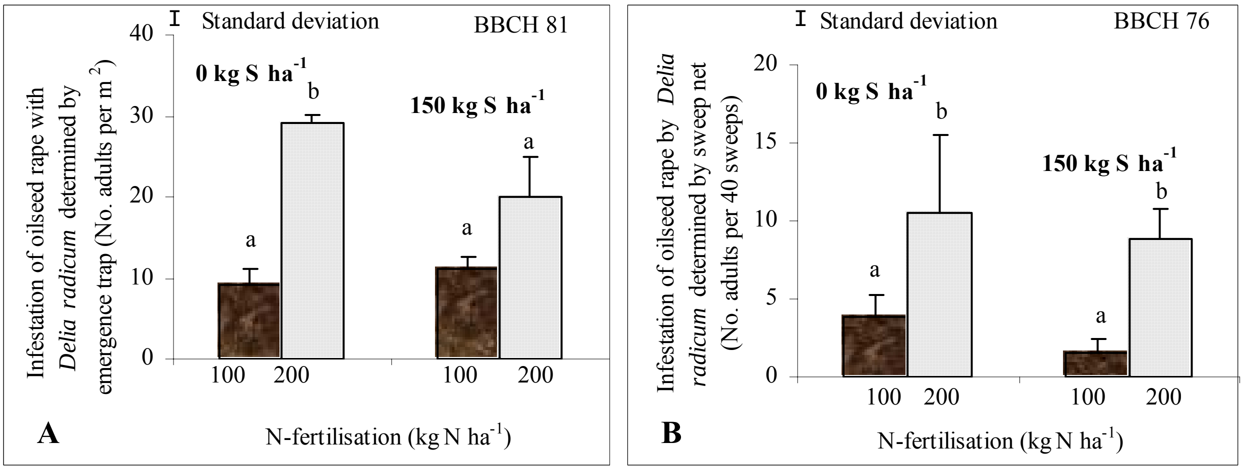


Fig. 3.34: Number of adults of *Delia radicum* which were collected by sweep net (B) and emergence traps (A) at different growth stages of oilseed rape (var. Lion) in relation to N-fertilisation in 2005 under different S-supply (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

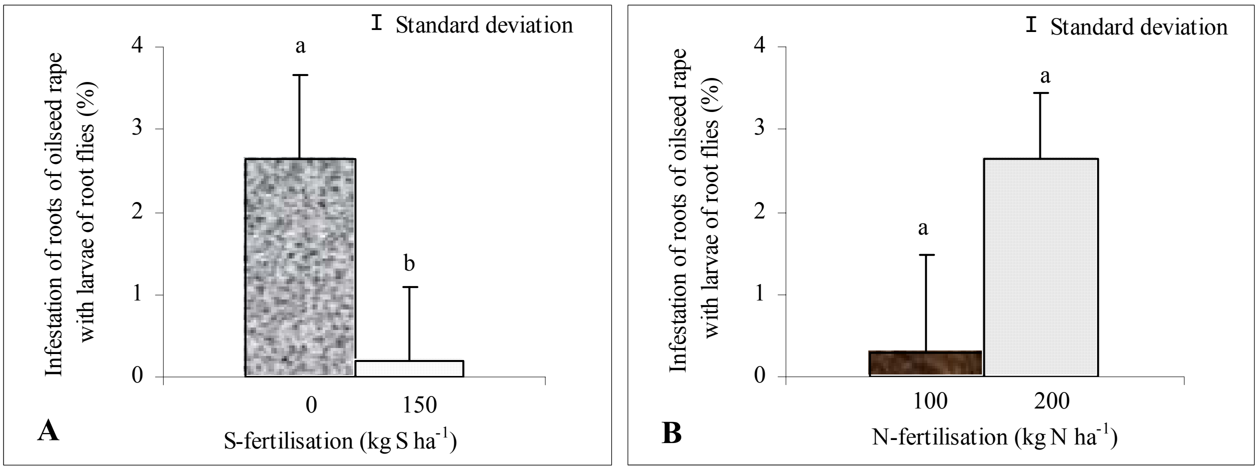


Fig. 3.35: The percentage of roots of oilseed rape which were infested with larvae of root flies in relation to S-fertilisation (A) and N-fertilisation (B) in early crop development (BBCH 13-14) (different letters denote to significant differences between treatments at the 0.05 level by the U-test) (season 2004/2005).

II Effect of S- and N-fertilisation on the infestation with adults of Delia platura

Adults of *Delia platura* were collected by sweep net at different growth stages. No consistent results were observed in 2004 for the population dynamic of adults of *Delia platura* in relation the S-supply. Higher numbers of *Delia platura* adults were collected from S-unfertilised plots of oilseed rape of the variety Lipton (Fig. 3.36) (A) while for the variety Bristol a higher infestation was observed in plots which received an S-application (Fig. 3.36) (B).

In 2005, S-application significantly decreased the infestation with adults of *Delia platura* before flowering at BBCH 50, at full flowering (BBCH 64) and at the beginning of pod ripening (BBCH 82) (Fig. 3.37) (A).

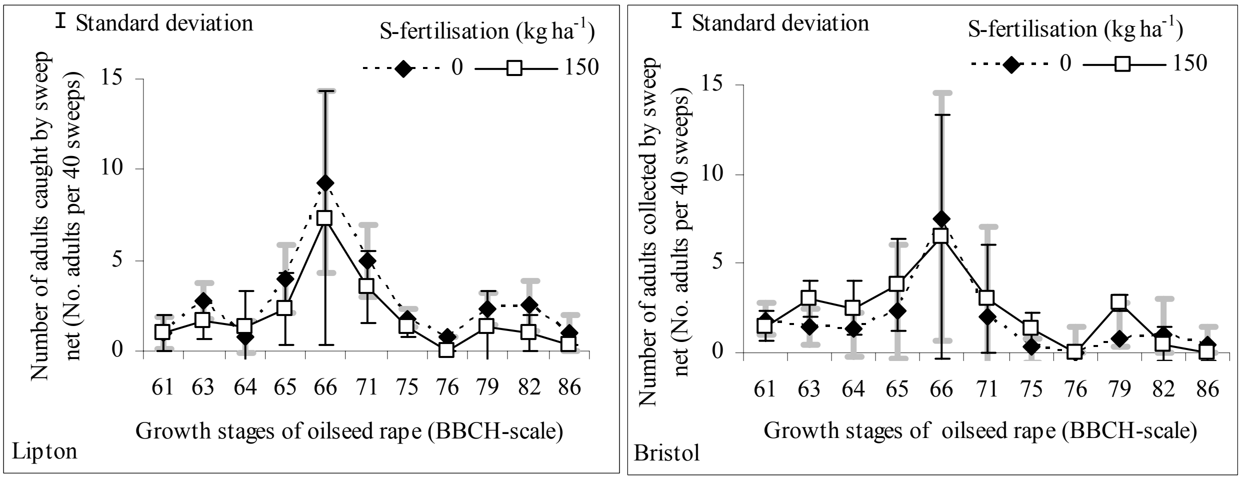


Fig. 3.36: Influence of S-fertilisation on the infestation of different varieties of oilseed rape with adults of *Delia platura* during different growth stages in 2004.

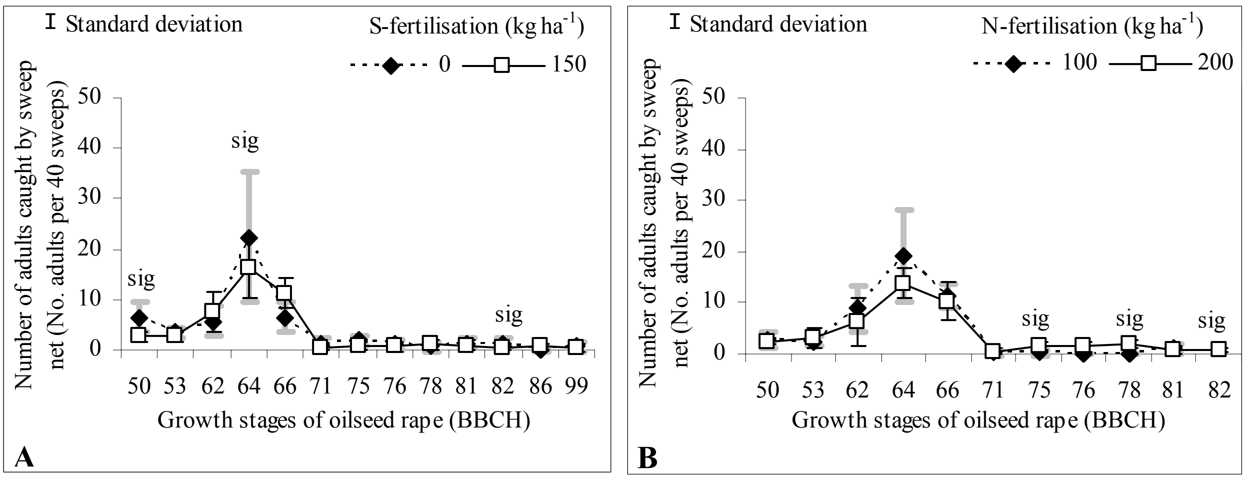


Fig. 3.37: Influence of S-fertilisation (A) and N-fertilisation (B) on the infestation with adults of *Delia platura* during different growth stages of oilseed rape in 2005 (sig. denote to significant differences between treatments at the 0.05 level by the U-test).

In the beginning of crop growth until pod development there was no significant difference in the infestation of oilseed rape with *Delia platura* in relation to the N-supply. Later during pod development a significantly higher infestation with adults of *Delia platura* was observed in plants which were fertilised with the higher dose of N (200 kg N ha^{-1}) (Fig. 3.37) (B).

Only a low number of adults of the turnip root fly (*Delia florilega)* was collected during all growth stages of the plant. Therefore only the total hatching number was tested over the whole season in relation to S-fertilisation. No significant differences were observed in relation to S- and N-fertilisation (Table A.31) but these results can not be generalised as the number of collected individuals was to low and differences are probably only relevant at certain growth stages of oilseed rape.

III Effect of S- and N-fertilisation on the occurrence of adults of the leaf miner fly (Scaptomyza flava)

The results show that the relative infestation rate with adults of *Scaptomyza flava* is increasing with S-fertilisation (Table A.32). An increasing population of *Scaptomyza flava* with S-fertilisation was observed over the whole vegetation period of oilseed rape in 2004 and the result was confirmed by different trapping methods. Also in 2005 the population of *Scaptomyza flava* was positively affected by S-fertilisation especially during early leaf development (BBCH 17, 19), full flowering (BBCH 66) and during pod ripening (BBCH 83, 86, 99) (Table A.32).

A different trend was found when adults were collected by suction trap during different growth stages. Significantly higher numbers of adults were collected from S-unfertilised plots by suction trap during the two peak times of occurrence of *Scaptomyza flava* (Fig. 3.38).

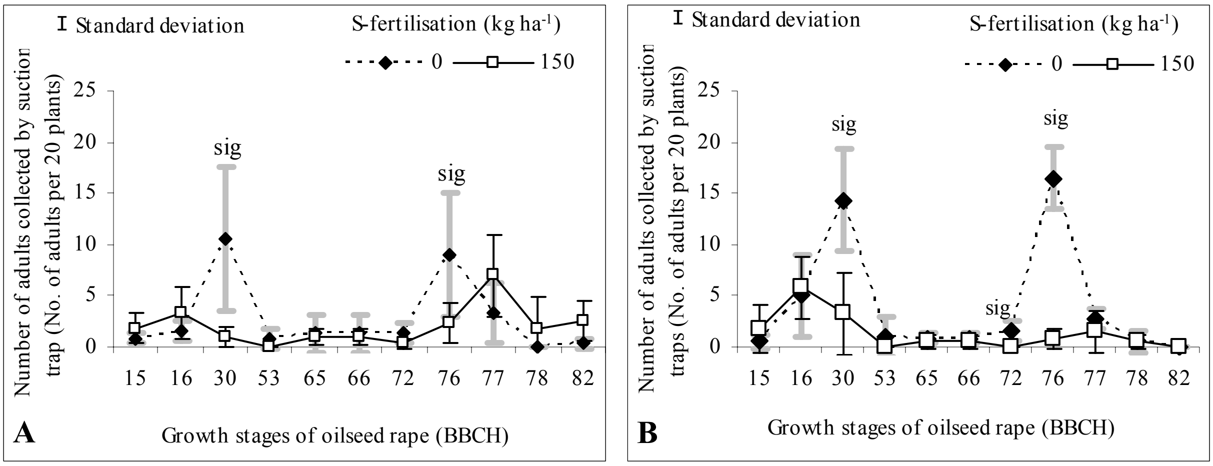


Fig. 3.38: Effect of S-fertilisation on the population dynamic of adults of *Scaptomyza flava* collected by suction trap during different growth stages of oilseed rape which received 100 kg N ha^{-1} (A) and 200 kg N ha^{-1} (B) in 2005 (sig. denote to significant differences between treatments at the 0.05 level by the U-test).

The results in figure 3.38 also reveal the positive response of *Scaptomyza flava* adults to N-application during different growth stages. Significantly higher numbers of adults were captured from oilseed rape plants that received 200 kg N ha^{-1} compared to the lower dose of only 100 kg N ha^{-1}. This positive effect was highest at the peak of occurrence at early crop development (BBCH 30) as figure 3.38 was shown and at full pod development (BBCH 76) (Fig. 3.39) (B). Moreover sampling of the new generation of adults by emergence traps revealed that there were two peaks of occurrence (BBCH 86, 100) were a clear positive effect of N-application on the emergence of *Scaptomyza flava* were found (Fig. 3.39) (A).

In conclusion, different species of root flies seemed to response differently to S-fertilisation. S-fertilisation increased the population of *Delia radicum*, while the population of *Delia platura* was negatively affected by S-fertilisation at most growth stages of oilseed rape (Fig. 3.40). Also *Scaptomyza flava* as leaf miner fly was negatively affected by S-supply as was shown in fig. 3.40.

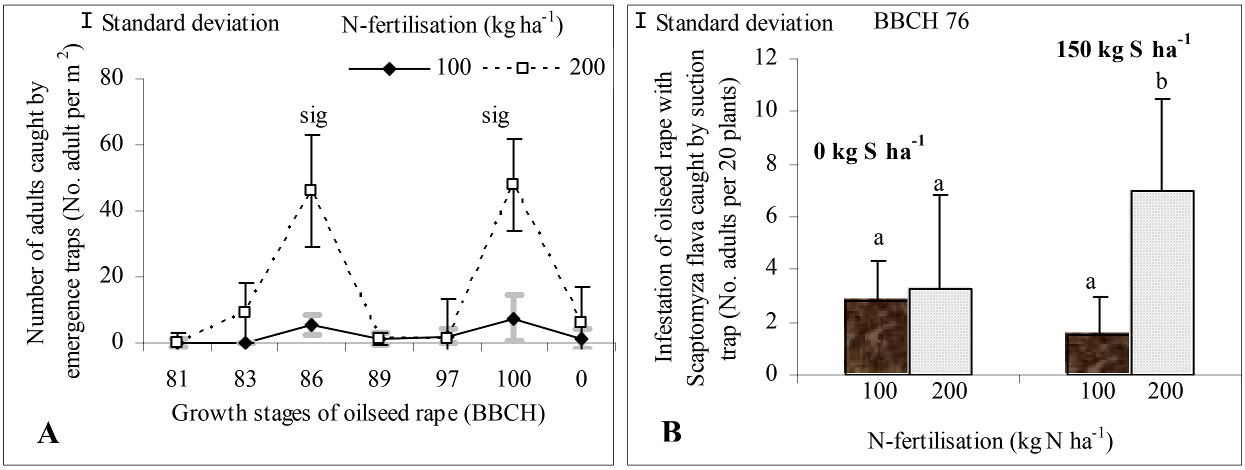


Fig. 3.39: Effect of N-application on the infestation of oilseed rape (var. Lion) with adults of *Scaptomyza flava* during different growth stages of oilseed rape in 2005 (adults were collected by emergence traps (A) and suction trap (B)) (different letters and sig. denote to significant differences between treatments at the 0.05 level by the U-test).

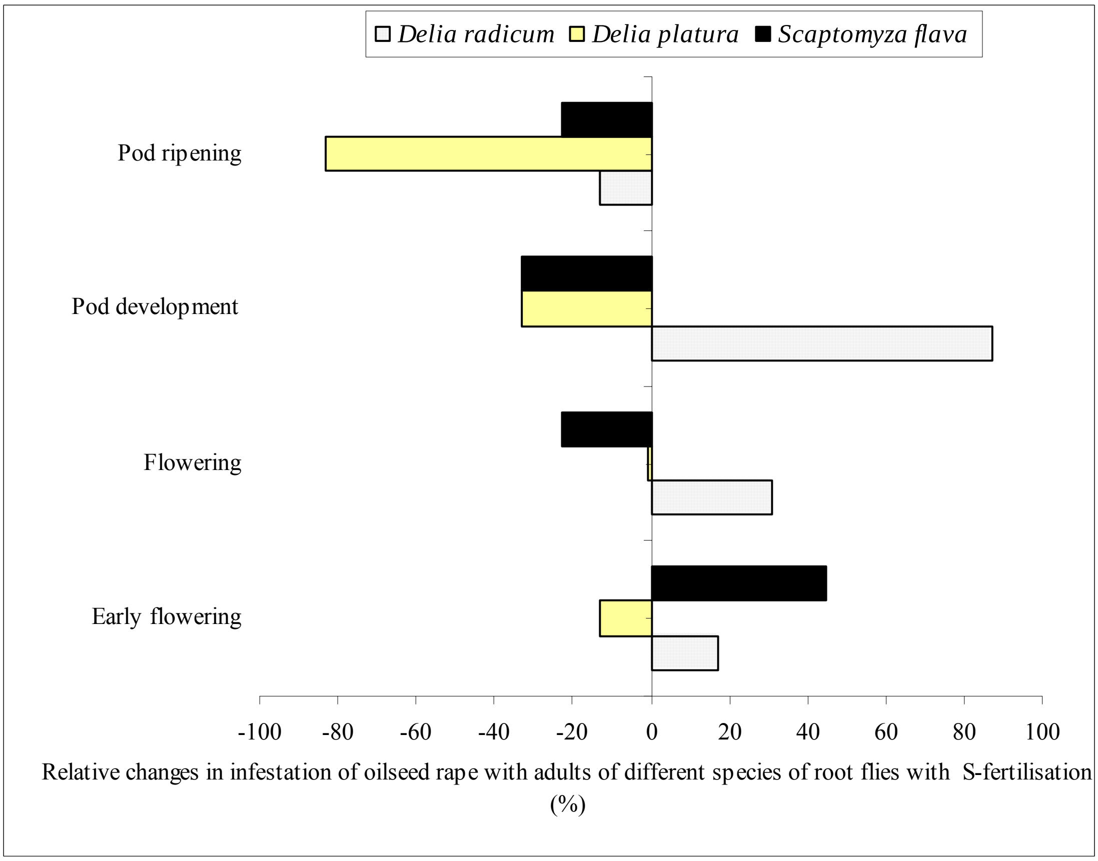


Fig. 3.40: Relative changes in the infestation level with adults of different species of root flies and *Scaptomyza flava* relative to S-fertilisation in 2004 at different growth stages of oilseed rape.

3.8 *Influence of S- and N-fertilisation on the number of cabbage aphid (Brevicoryne brassicae)*

The results show that a higher number of *Brevicoryne brassicae* was collected from S-fertilised plants (Fig. 3.41). The infestation rate with *Brevicoryne brassicae* relative to S-fertilisation over the whole season (from flowering to ripening) was listed in table A.33.

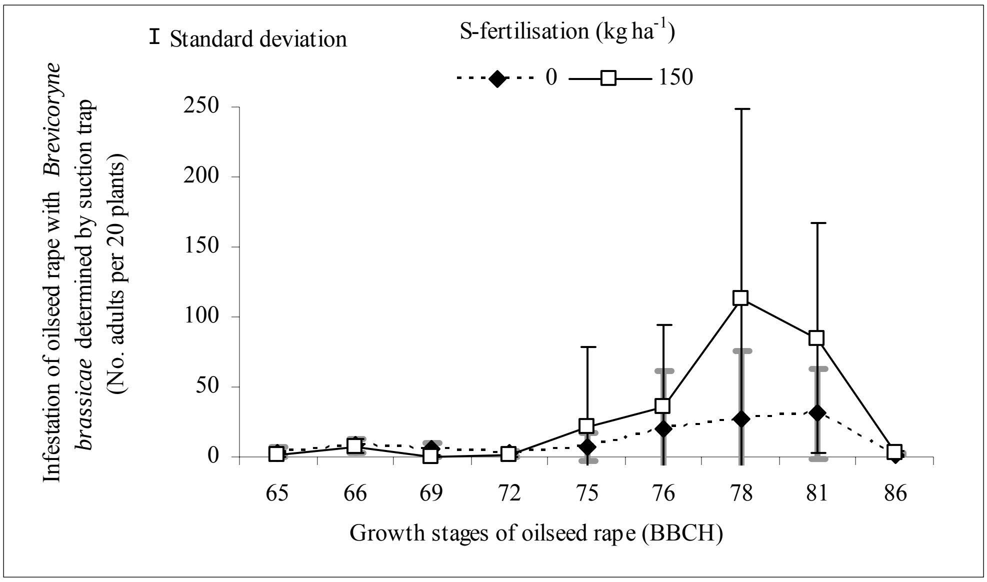


Fig. 3.41: Effect of S-fertilisation on the infestation of oilseed rape with adults of *Brevicoryne brassicae* collected by suction trap during different growth stages of oilseed rape in 2004.

Also in 2005 *Brevicoryne brassicae* showed a positive response to S-fertilisation. A significant higher number of *Brevicoryne brassicae* was collected from plots which received an S-application by emergence traps (Fig. 3.42) (A). At the end of pod development (BBCH 78) and at the beginning of pod ripening (BBCH 83) (Fig. 3.43) higher numbers of *Brevicoryne brassicae* were collected by suction trap on plots with S-fertilisation. N-fertilisation was also positively influencing the population dynamic of *Brevicoryne brassicae* (Fig. 3.42) (B).

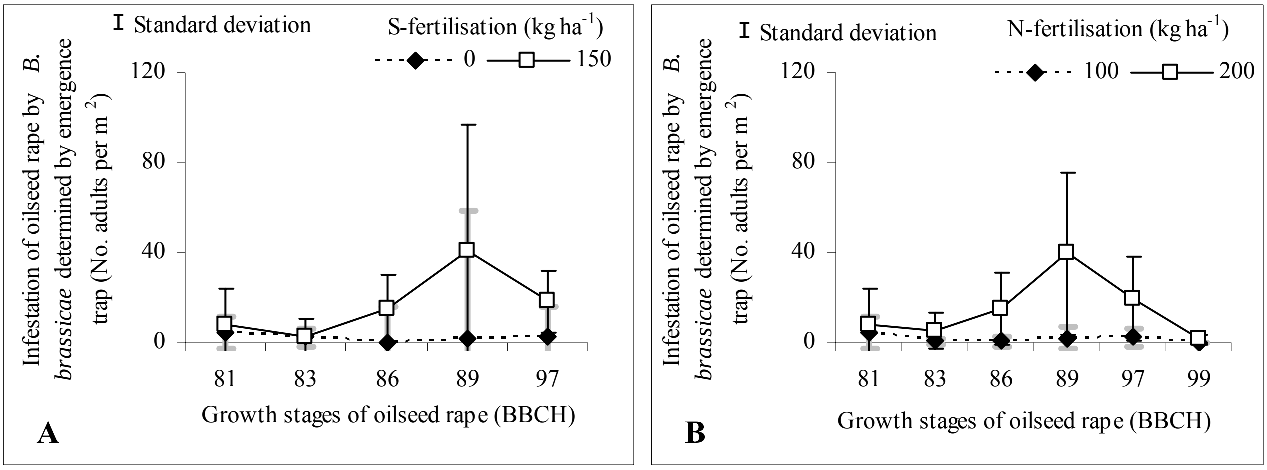


Fig. 3.42: Adults of *Brevicoryne brassicae* which were collected by emergence traps in relation to S- fertilisation (A) and N-fertilisation (B) during different growth stages of oilseed rape in 2005.

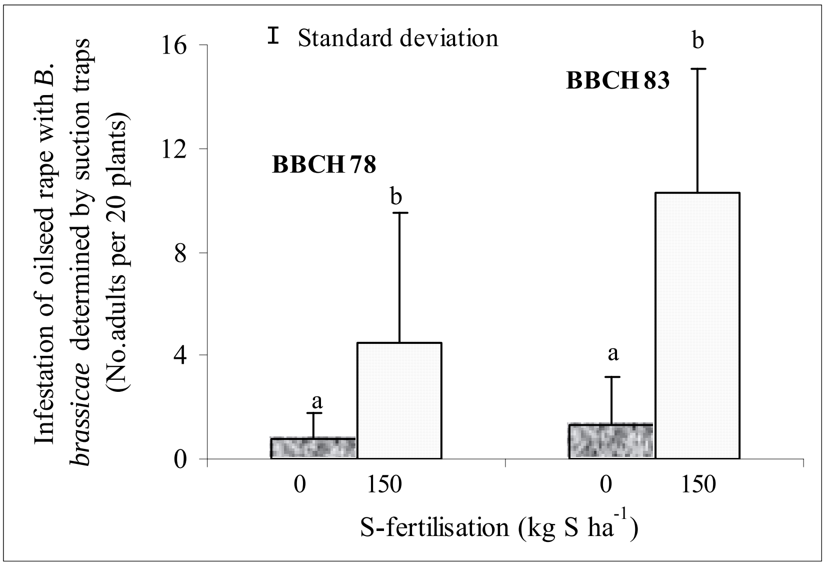


Fig. 3.43: Number of adults of *Brevicoryne brassicae* which were collected by suction trap in relation to S-fertilisation during different growth stages of oilseed rape (var. Lion) in 2005 (different letters denote to significant differences between S-treatments at the 0.05 level by the U-test).

3.9 Influence of S- and N-fertilisation on the occurrence of Staphylinidae and Tachyporus (adults and larvae)

In this chapter, the results of the investigation on predacious insects are shown. The occurrence of adults and larvae of the *Staphylinidae* family and its genus *Tachyporus* which were also collected by different traps is shown in relation to S- and N-fertilisation.

I Influence of S- and N-supply on number of adults and larvae of Staphylinidae family

The rove beetle *Staphylinidae* is a polyphagous predator of different oilseed rape pest species and members of the subfamily *Staphylininae* feed on a wide range of hosts. Adults and larvae of *Staphylinidae* have an important function in controlling adults and larvae of many different pest species of oilseed rape. Full grown larvae of *Dasineura brassicae*, *Meligethes* spp. and various species of *Ceutorhynchus* weevils are most likely preys for *Staphylinida*e (Büchs, 2003c). Therefore the relationship between *Staphylinidae* and their preys in relation to S-fertilisation was studied in this work.

In 2004, adults of the *Staphylinidae* were collected in relation to S-fertilisation from different oilseed rape varieties (Lipton and Bristol) with emergence traps. A significantly higher number of adults of *Staphylinidae* were captured from S-fertilised plants at full pod development (BBCH 74, 76) for the variety Bristol (Fig. 3.44) (B). A different trend was observed for the variety Lipton (Fig. 3.44) (A).

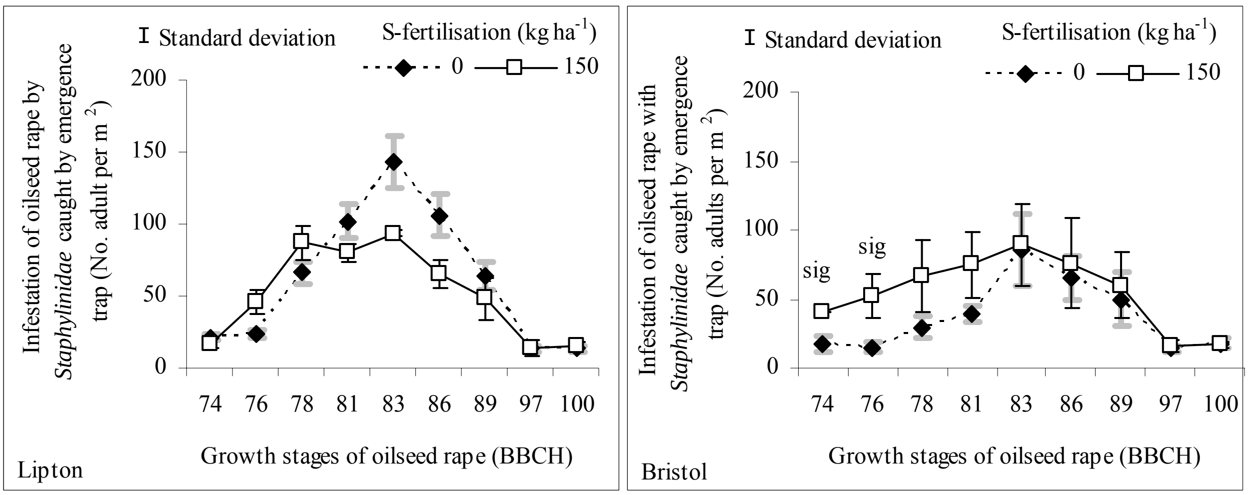


Fig. 3.44: Effect of S-fertilisation on the number of adults of *Staphylinidae* collected during different growth stages by emergence traps from oilseed rape variety in 2004 (sig. denote to significant differences between S-treatments at the 0.05 level by the U-test).

A positive response to high doses of N was found in 2005 while S-fertilisation decreased the population of *Staphylinidae* in that year (Fig. 3.45) (A). Significantly more adults of *Staphylinidae* were collected by emergence traps during pod ripening in the plots which received the higher N-application (Fig. 3.45) (B).

This study indicated that reproduction success of *Staphylinidae* was increased by S-fertilisation about 45% (from 0.33 to 0.48).

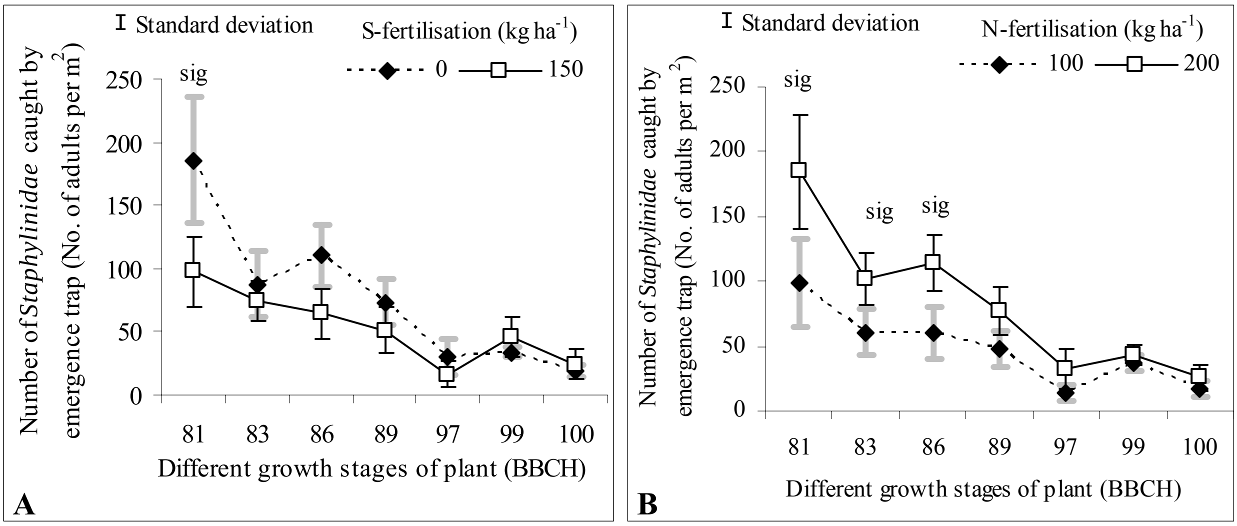


Fig. 3.45: Number of adults of *Staphylinidae* collected during different growth stages of oilseed rape (var. Lion) in relation to S-fertilisation (A) and N-fertilisation (B) in 2005 (sig. denote to significant differences between treatments at the 0.05 level by the U-test).

Larvae of the *Staphylinidae* were collected by funnel traps from pod development (BBCH 67) until harvest of the plant (BBCH 99) but only in 2005. S-fertilisation significantly decreased the number of predator larvae at the beginning of pod development and full pod development (BBCH 76) (Fig. 3.46). This decrease may be caused by a decrease in number of their preys such as larvae of *Meligethes* spp. what will be discussed later. N-fertilisation had no significant influence on the number of collected larvae but the number of larvae tends to be higher in plots with lower N-application (Fig. 3.46 A).

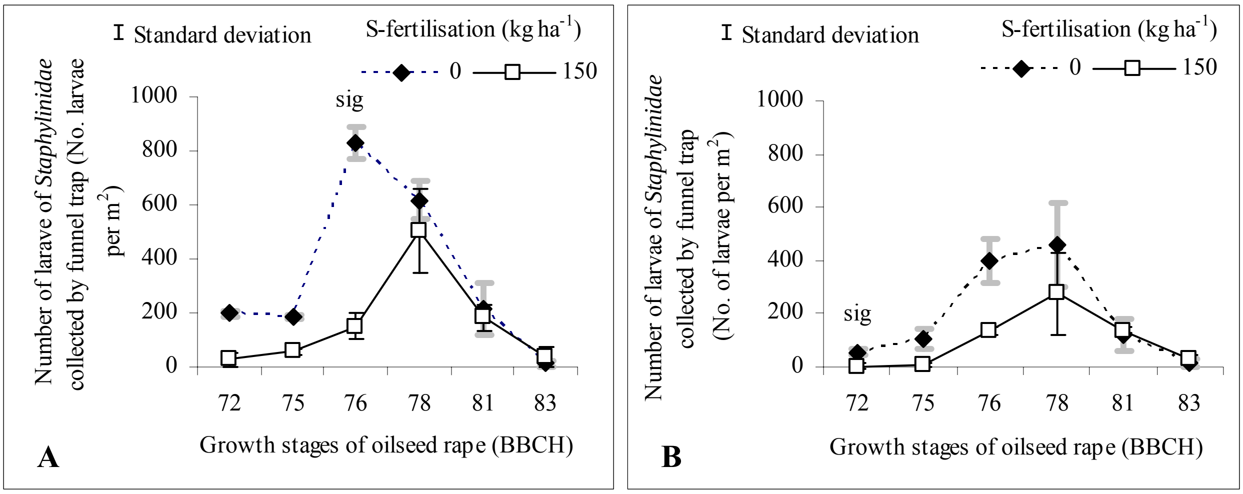


Fig. 3.46: Effect of S-fertilisation of oilseed rape (var. Lion) on the occurrence of larvae of *Staphylinidae* which were collected by funnel traps under different N-supply in 2005 (A: low dose of N (100 kg N ha^{-1}) B: high dose of N (200 kg N ha^{-1}) (sig. denote to significant differences between S-treatments at the 0.05 level by the U-test).

II Influence of S- and N-supply on the number of adults and larvae of Tachyporus genus

Adults and larvae of *Tachyporus* predator were investigated in this work as genus of the *Staphylinidae* family which is active during pod development and pod ripening. This predator is a suspected substantial predator of larvae of *Meligethes* spp. in oilseed rape (Schlein and Büchs, 2004).

In 2004 as well as in 2005 S-fertilisation decreased the number of adults of *Tachyporus* which were collected by emergence traps (Table A.35). Significantly higher number of adults were collected by emergence traps from S-unfertilised plots during the different pod ripening stages (BBCH 81, 83, 86, 89) (Fig. 3.47). A significantly higher number of *Tachyporus* larvae were determined in S-unfertilised plants when 50-60% of pods were developed (BBCH 75, 76) and over the whole period of pod development and ripening (Fig. 3.48) (A). The results also revealed higher number of *Tachyporus* larvae in oilseed rape plants that received the lower dose (100 kg N ha^{-1}) of N (Fig. 3.48) (B). The population dynamic in relation to that of potential prey organisms is discussed in chapter 4.4.

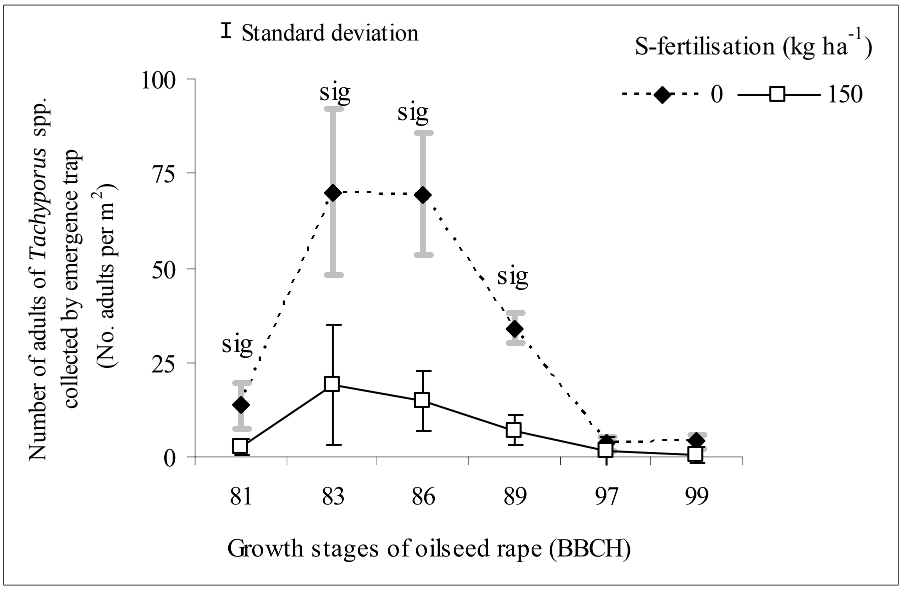


Fig. 3.47: Number adults of *Tachyporus* collected by emergence traps during different growth stages of oilseed rape in relation to the S-supply in 2004 (sig. denote to significant differences between treatments at the 0.05 level by the U-test).

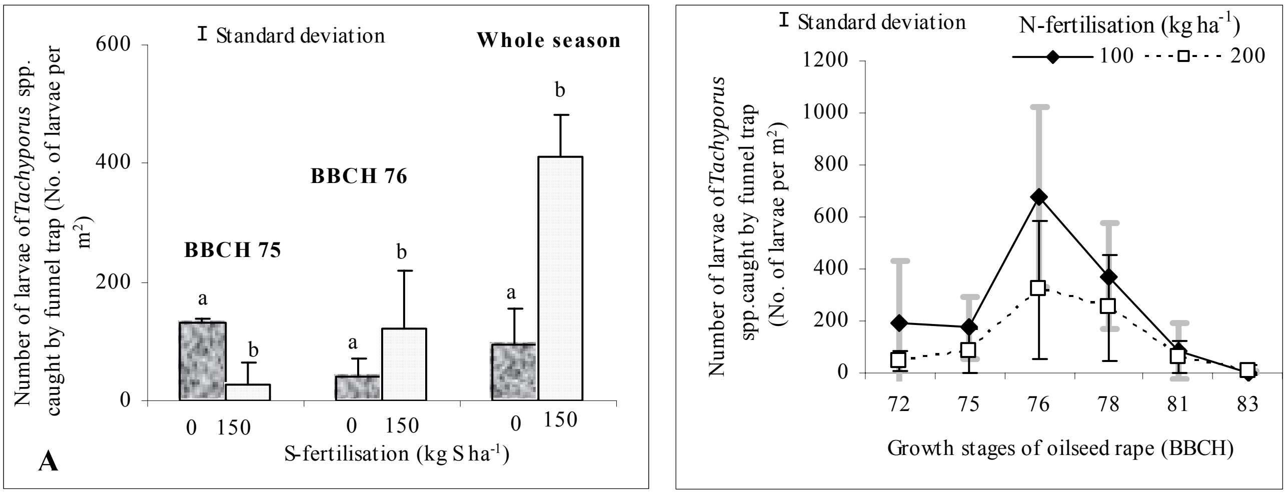


Fig. 3.48: Effect of S-fertilisation (A) and N-fertilisation (B) on the number of *Tachyporus* spp. larvae collected by funnel traps during different growth stages of oilseed rape in 2005 (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

III Interaction between predators (Staphylinidae and *Tachyporus) and their preys*

The relationship between *Staphylinidae* and their preys in relation to S-application was studied. In 2004, the population of adults of *Delia radicum,* for example, was positively affected by S-fertilisation at pod development, and at the same time, the population of *Staphylinidae* increased (Fig. 3.49) and a significant positive correlation ($r^2 = 0.56$, $P < 0.05$) was found between the number of adults of *Delia radicum* and *Staphylinidae* at pod ripening. Also appearance of adults of the new generation of *Staphylinidae* coincided with the time when the fully grown larvae of the second generation of *Dasineura brassicae* drop down to the soil. The larval population of *Dasineura brassicae* increased with S-fertilisation (Fig. 3.50) and therefore also an increase in the population of *Staphylinidae* with S-fertilisation was observed.

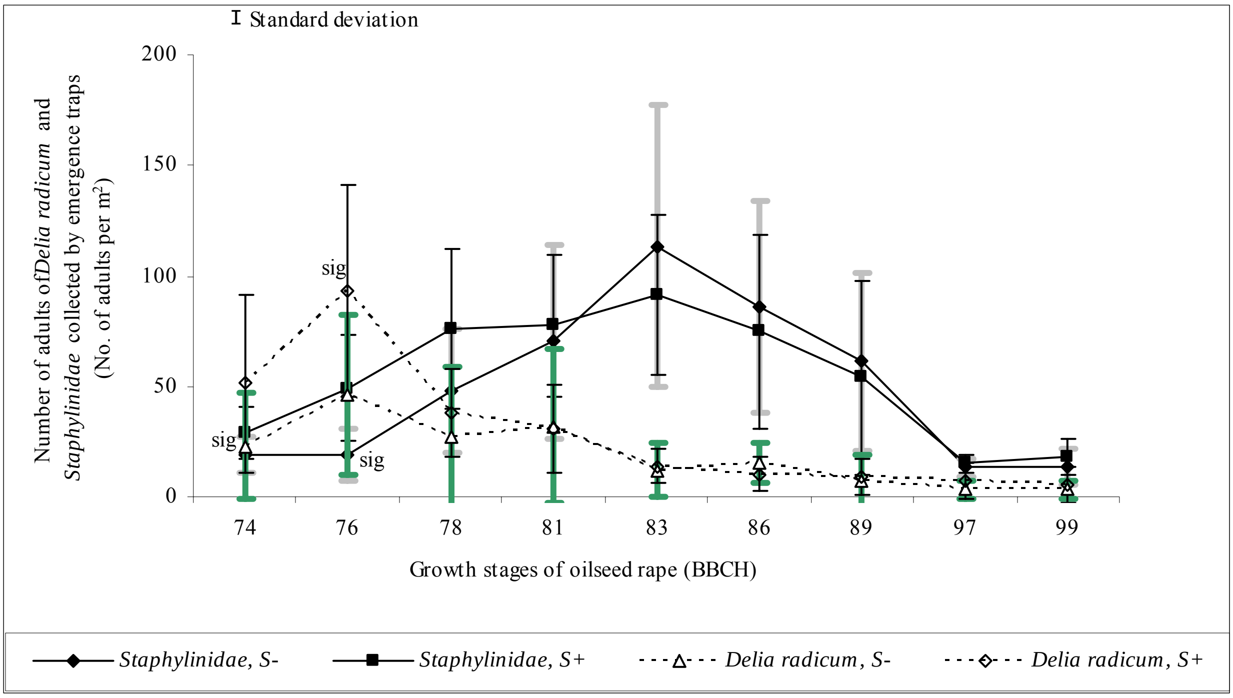


Fig. 3.49: Relationship between the population of adults of *Delia radium* and their predators *Staphylinidae* in relation to S-nutrition during pod development and pod ripening of oilseed rape in 2004 (S-: plots without S-application, S+: plots which received 150 kg S ha^{-1}).

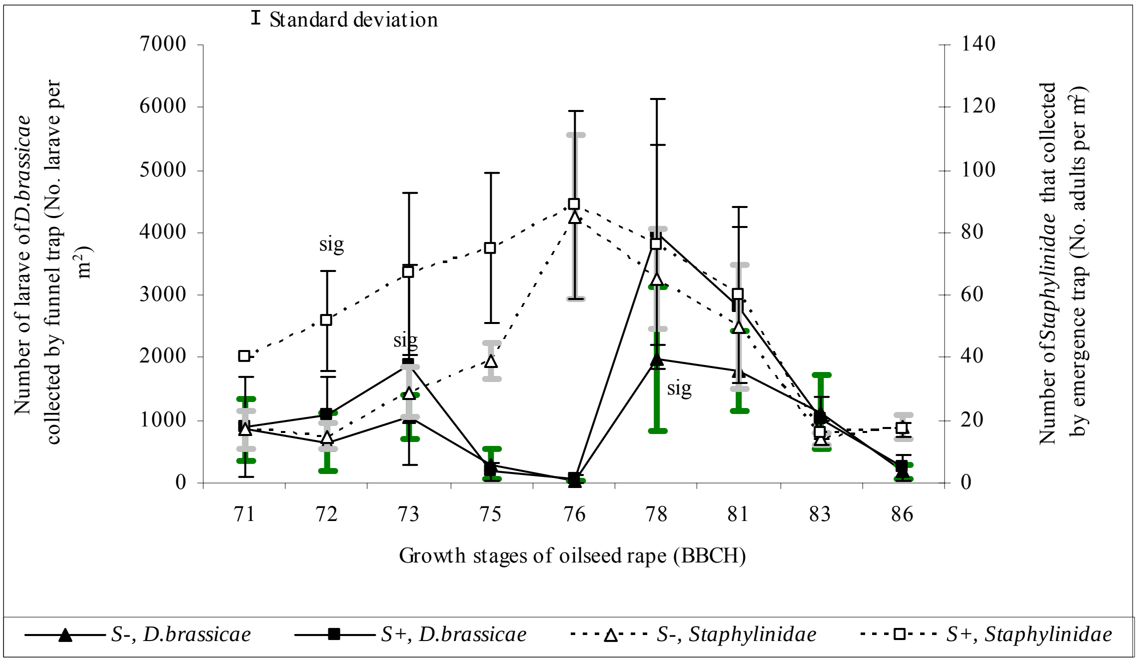


Fig. 3.50: Interaction between larvae of *Dasineura brassicae* and their predator the adults of *Staphylinidae* in relation to S-fertilisation in oilseed rape (var. Bristol) in 2004 (S- plots without S-application, S+ plots which received 150 kg S ha^{-1}).

In 2005 the quantitative relation of rove beetle larvae (*Staphylinidae* and *Tachyporus*) and *Meligethes* spp. larvae has been recorded with funnel traps. The number of larvae of the *Staphylinidae* family was lower in plots that received a S-fertilisation, especially when also N was applied in the higher dose during whole pod development (from BBCH 71 until BBCH 81). The response of *Meligethes* spp. larvae to S-fertilisation was different between different growth stages. Higher numbers were captured from S-unfertilised plots at BBCH 71-72 while during full pod development, and afterwards, (BBCH 75, 76, 78 and 81), higher numbers were collected in S-fertilised plots. This change can be caused by the relationship between *Meligethes* spp. larvae and their predator larvae. A higher number of predator larvae should be related to a high presence of their food source (*Meligethes* spp. larvae) in plants.

At BBCH 71 and 72 significantly more larvae of *Meligethes* spp. were collected from plots which received no S-fertilisation and the lower dose of N-application. Also larvae of the *Staphylinidae* were collected in higher numbers from plots without S-fertilisation and with an increasing population of *Staphylinidae* the number of *Meligethes* spp. larvae decreased rapidly (Fig. 3.51).

The same was observed when the relationship between *Meligethes* spp. larvae and *Tachyporus* larvae was studied over the growing season of oilseed rape in relation to S-fertilisation (Fig. 3.52).

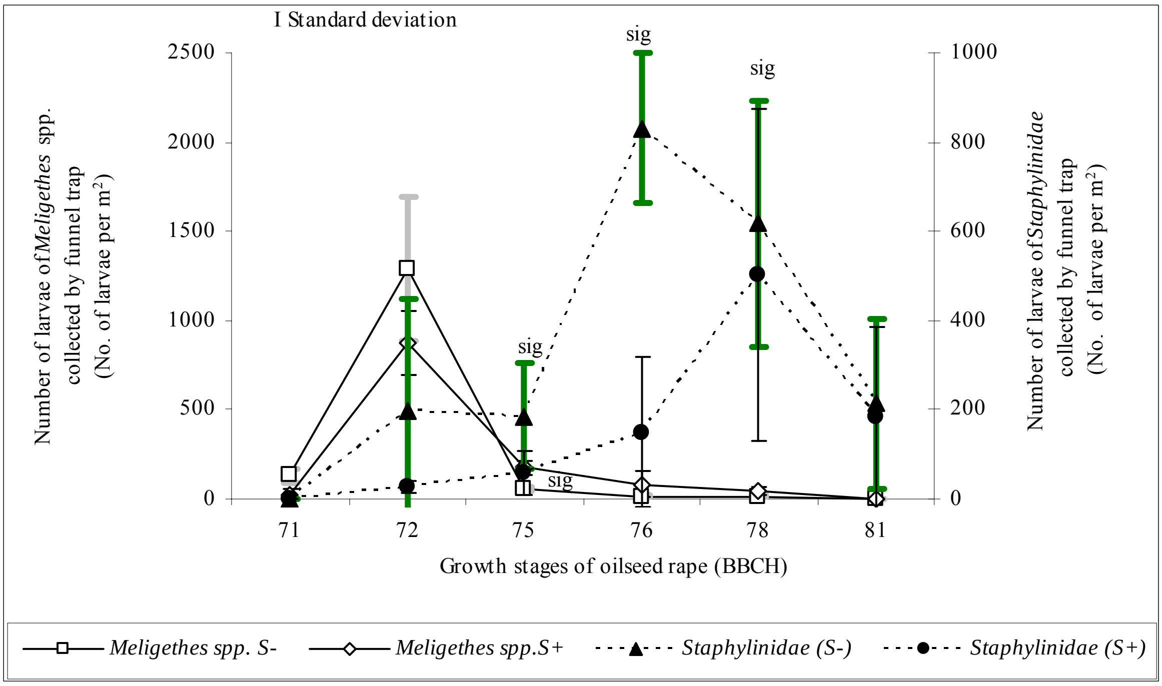


Fig. 3.51: Interaction between *Meligethes* spp. larvae and their predator *Staphylinidae* larvae in relation to S-fertilisation (data from 2005) (S-: plots without S-application, S+: plots which received 150 kg S ha^{-1}).

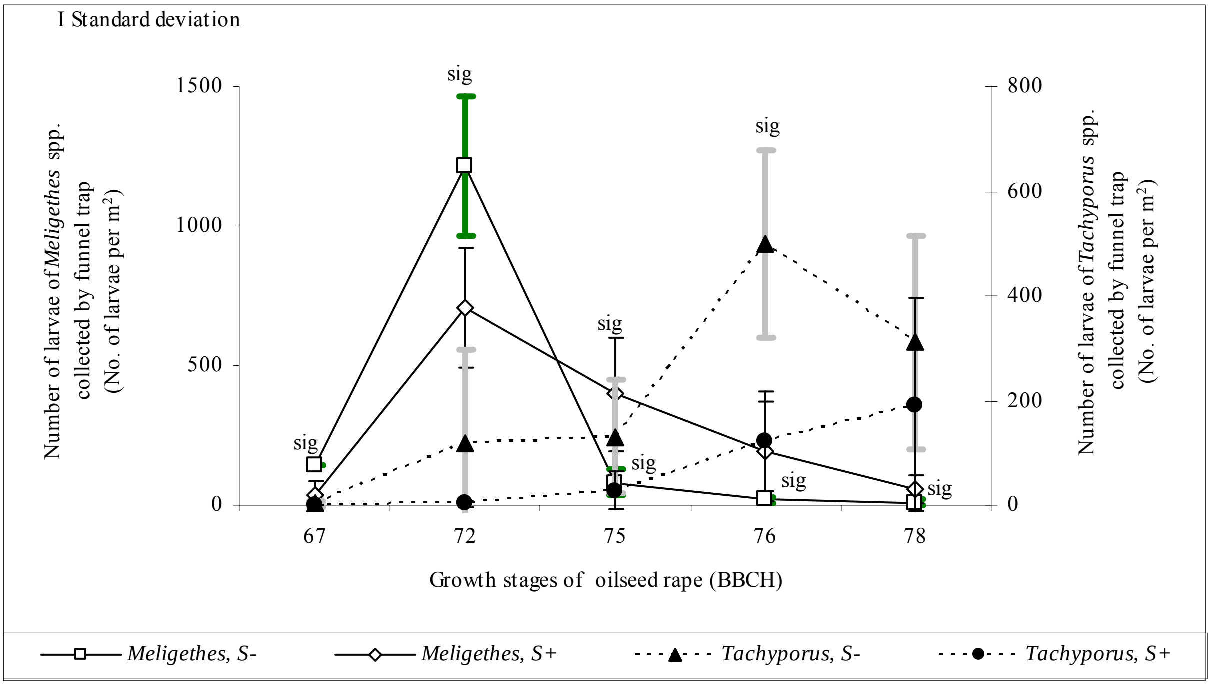


Fig. 3.52: Interaction between *Meligethes* spp. larvae and their predator *Tachyporus* larvae in relation to S-fertilisation (data from 2005) (S-: plots without S-application, S+: plots which received 150 kg S ha^{-1}).

This relationship between *Meligethes* spp. larvae and the different predator larvae point out that the influence of the S-nutritional status on the population dynamic can be overlaid by other relationships such as the population dynamic of predator larvae. Therefore it is not useful to discuss for example the population dynamic of *Meligethes* spp. larvae after BBCH 71 in relation to the S-nutritional status because other factors are than more important what is shown in figure 3.51 and 3.52.

3.10 Influence of S-fertilisation of oilseed rape on the number of miscellaneous insects

The aphidophagous hover fly is an important predator in oilseed rape cropping because the hover fly larvae can effectively regulate aphid infestations. Adults of *Syrphidae* predators were collected by sweep net from inflorescence emergence until the harvest of oilseed rape in 2005. Significantly higher numbers of adults were collected from S-unfertilised plots when the flower pods were present but still enclosed by leaves (BBCH 50), at flowering (BBCH 62, 64, 66) and in medium over the whole season (Fig. 3.53). The appearance of aphids on oilseed rape was observed usually later at pod ripening when no influence of S-fertilisation on predators was found. No close relationship was found between the population dynamics of aphids and their predators (Fig. 3.54).

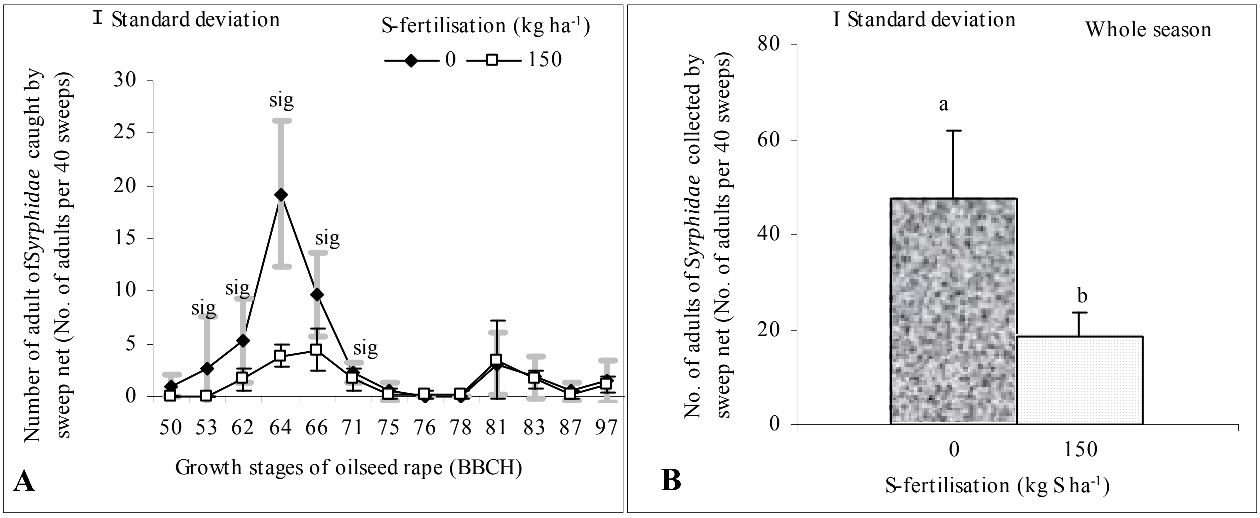


Fig. 3.53: Effect of S-nutrition on the occurrence of adults of *Syrphidae* collected by sweep net during different growth stages of oilseed rape (A) and over the whole season 2004/2005 (B) (different letters and sig. denote to significant differences between S-treatments at the 0.05 level by the U-test).

Adults of Thrips were also collected with different traps during different growth stages of the plants but no significant differences were found in relation to S-fertilisation (Table A.37).

Spiders are polypredator and they are affected by several factors such as season and location because these factors determine the composition of their preys (Büchs, 2003c). The population of *Brevicoryne brassicae,* which is one important food source of spiders, was not the only factor which was affecting the spider population. However the peak occurrence of *Brevicoryne brassicae* and spiders coincided at full pod development (BBCH 76) (Fig. 4.55) where spiders showed a negative response to S-fertilisation.

The results show that the composition of oilseed rape visiting insects is not only directly influenced by the S-nutrition but also indirectly with view to the predator insects which population depend on the occurrence of pest organisms.

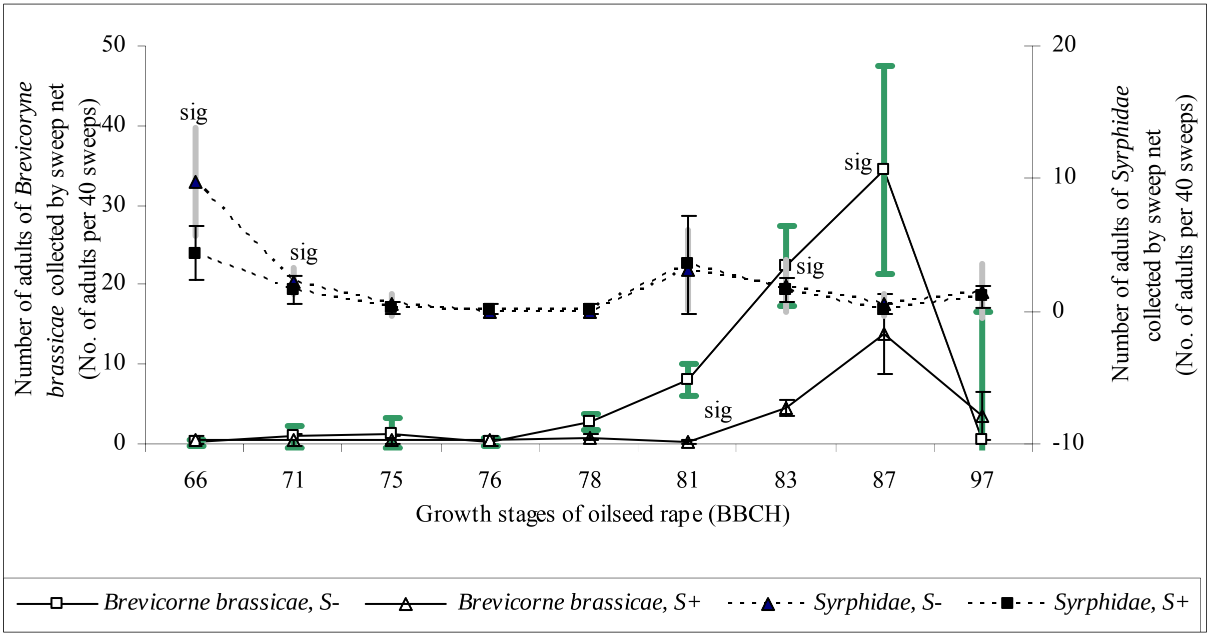


Fig. 3.54: Interaction between adults of *Brevicoryne brassicae* and their predator adults of *Syrphidae* in relation to S-fertilisation in 2005 (S-: plots without S-application, S+: plots which received 150 kg S ha^{-1}).

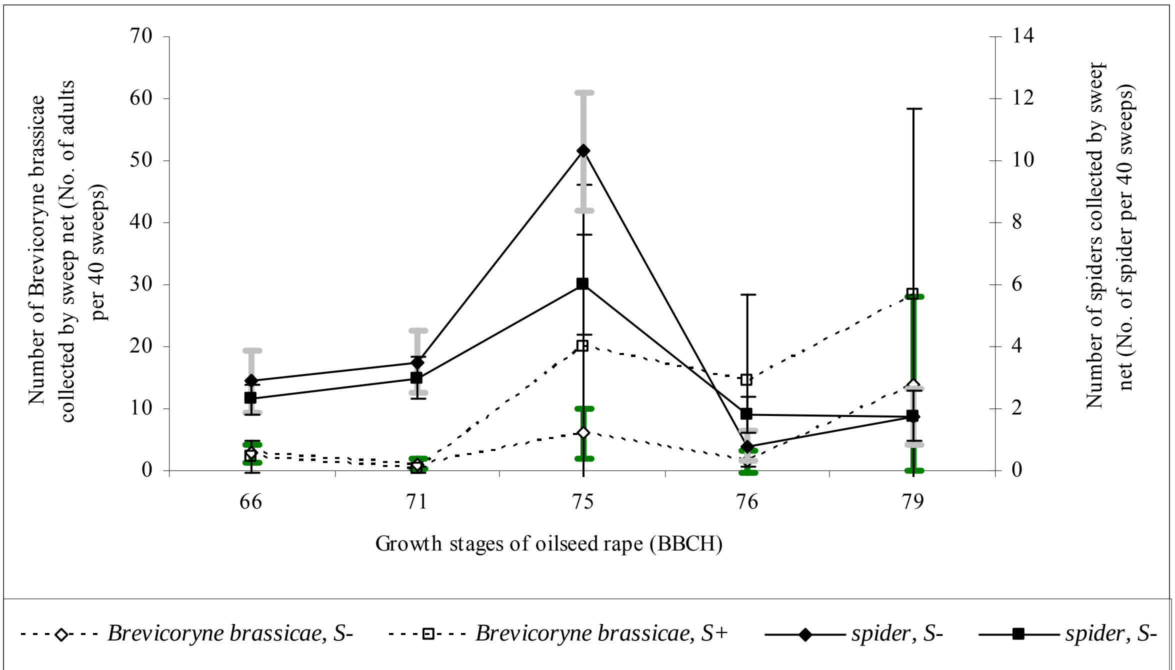


Fig. 3.55: Relationship between Spiders and *Brevicoryne brassicae* collected by sweep net from oilseed rape (var. Bristol) in relation to S-fertilisation in 2004.

Despite of the fact that it was no special aim of this study to investigate differences in the occurrence of different insect species in relation to oilseed rape variety same differences were found which are summarised in figure 3.56. Here a comparison for the varieties Lipton and Bristol is shown as both varieties were grown in the same trial in 2004 and had the same climatic conditions and surrounding landscape. In figure 3.56 the relative infestation of the variety Bristol is shown relative to the variety Lipton for the different oilseed rape visiting pest organisms at their most relevant growth stage of the crop. Most oilseed rape visiting insects preferred to feed on Bristol and only the relative infestation with *Dasineura brassicae* was by 13% lower than for the variety Lipton. Therefore the variety of oilseed rape seems to be another factor which can have an influence on the infestation of oilseed rape with pest organisms.

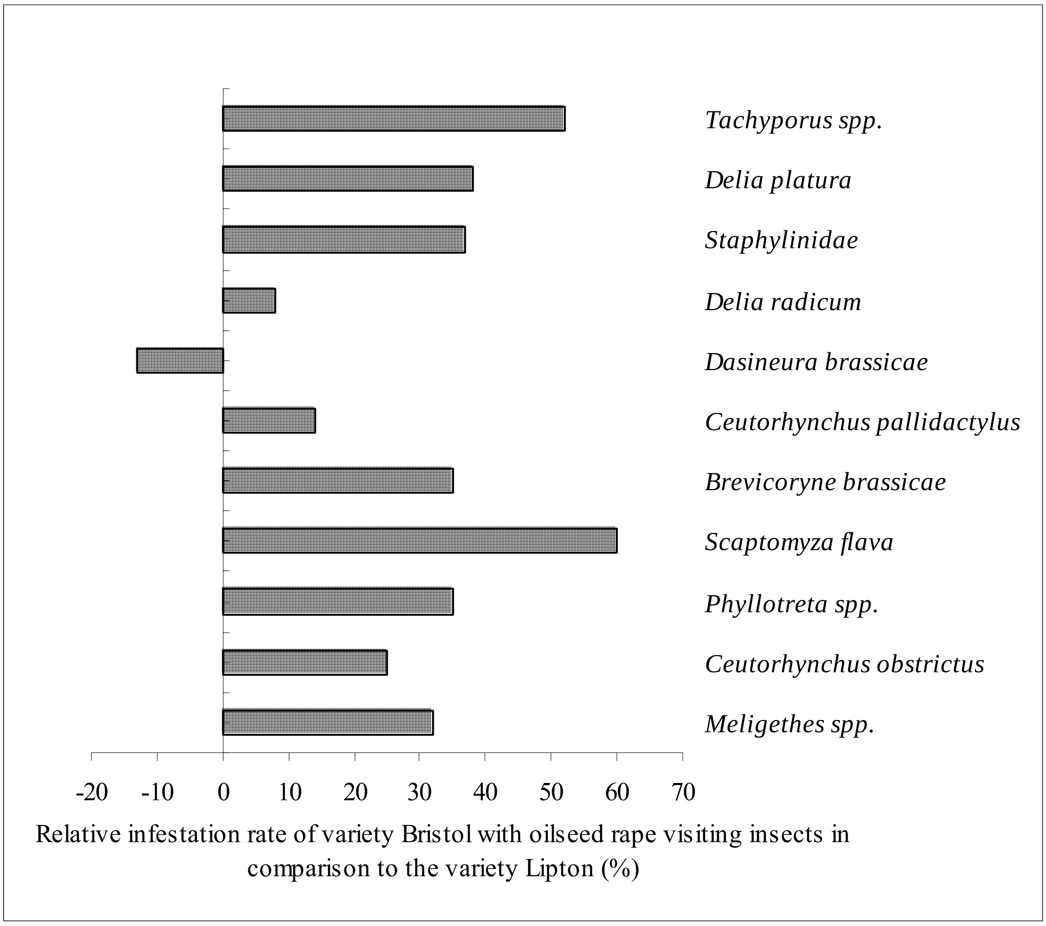


Fig. 3.56: Relative infestation rate of the variety Bristol in comparison to Lipton with different oilseed rape visiting insects at different growth stage of oilseed rape (BBCH 66 for *Dasineura brassicae* and *Brevicoryne brassicae*, BBCH 78 for *Staphylinidae,* BBCH 81 for *Tachyporus* and BBCH 61-63 for other insect species) (data from 2003/2004).

4 Discussion

The reduction of atmospheric S-pollution in the last decade caused S-deficiency in different agricultural crops, especially in oilseed rape. S-deficiency not only had a negative impact on quality and yield of oilseed rape but can also affect the susceptibility of plants to certain insect species. However, only a very limited number of studies have focussed on the influence of S-fertilisation on S-concentration and secondary plant metabolism in oilseed rape in relation to infestation of oilseed rape plants with different insect species (pests and predators) during different growth stages. It was the aim of this study to elucidate the influence of S-nutrition on the infestation of oilseed rape with numerous insects. Two different experiments were conducted in 2004 and 2005 which differed in the size of the plots, the distance between S-fertilised and unfertilised plots, the variety of oilseed rape and also the surrounding landscape. Therefore the discussion of this thesis starts by studying the significance of experimental conditions on infestation of oilseed rape with insects (Chapter 4.1). In the following two chapters, the relationship between the S-nutritional status of oilseed rape and visiting insects is discussed. In chapter 4.2 the relationship between the S-nutritional status and different insect species is reported and in chapter 4.3 the interaction between insect species in relation to the S-nutrition is discussed. Moreover in chapter 4.4 the relationship between N-fertilisation and infestation of oilseed rape with different pests and beneficial insects is discussed and in the end of the discussion the possibility to use the S-nutritional status of oilseed rape to control infestation with special insects is controversially discussed for generalist and specialist insects of oilseed rape.

4.1 Significance of experimental conditions on infestation of oilseed rape with insects

The experimental conditions are of great significance for the infestation of oilseed rape with different insect species because seed density, variety of oilseed rape, crop rotation, surrounding landscape and many other factors are important for the quality of habitat and the source of food as well as for hibernation (Schmidt, 2004). Moreover abiotic factors like climatic conditions are of tremendous relevance like the strength of the winter or the temperature and humidity during spring and summer (Thomas *et al*., 2002). In the second experimental year the fields were treated with the usual insecticide program to get results under productional conditions which can be transferred directly to practice.

In 2004, the investigation was conducted in relatively small plots of 60 m^2 while in 2005 a plot size of 135 m^2 was chosen. The size of experimental plot is one factor that has an effect on the density, damage and distribution of arthropods. For example the width of plots shall be greater than the distance that individuals of insects might commonly move during a day (Prasifka and Hellmich, 2004). Therefore recommendations for an acceptable plot size may different for different insects (Prasifka *et al*., 2005). For example small plots are acceptable for insects which do not move very much such as mites but they are not suitable for flying insects that enter or leave experimental plots daily (Prasifka and Hellmich, 2004). Therefore, the plot size in the first year of experimentation was more suitable for some coleopteran beetle in comparison to flying insects.

Beside of the size of the plots also the distance between S-fertilised and unfertilised plots was different in both experiments. In 2004, the plots were directly side by side while in 2005 an experimental design with a distance of 200 m between the plots was performed. Generally, when plots are too close together the risk is higher that insects are attracted by a factor but also visit plants of the neighbouring plot. The percentage of migration from one plot to an other is different in relation to insect species. For example, the densities of *Phyllotreta* spp. decreased with increasing distance between different cropping treatments (Bergelson and Kareiva, 1987) while the Syrphids are not affected by distance (Hegland and Boeke, 2006). On the other hand a greater distance between the plots increase the risk that other factors like the surrounding landscape overlay the factor of interest. Another experimental factor which can have an influence on oilseed rape visiting insects is the variety of oilseed rape as it was found in present work (Fig. 3.56). The response of oilseed rape visiting insect species to cultivars of oilseed rape was different in relation to the growth stage of the plant, for example Büchi (1996) observed a relationship between the growth stage of the crop and egg laying of *Ceutorhynchus napi*. On average early varieties were less infested by *Ceutorhynchus napi*.

The surrounding landscape is an important experimental factor which has a significant influence on oilseed rape visiting insects too. The large-scale features are very important in determining the abundance and diversity of insects in oilseed rape. The landscapes in agricultural areas provide the favoured foraging and overwintering habitats to insects which affect the insect population (Clough, 2006; Thies *et al*., 2003; Schmidt, 2004).

In the first year of experimentation, the field was surrounded by a forest, while in the second year no forest but mainly cultivated land and orchards were close to the experimental site. The landscape structure can have an important influence of the total hatching number of oilseed rape visiting insects. For example Frank *et al.* (2006) found increasing numbers of *Dasineura brassicae* adults close to woodland. In general, the effect of landscape diversity on oilseed rape visiting insects is different for generalist and specialist species (Thies *et al*., 2003). The generalist insects feed on cruciferous as well as other plant families, therefore their abundance within a field increases with increasing landscape diversity (Thies *et al*., 2003; Jonsen and Fahrig, 2004). Complex landscapes with high habitat diversity can be expected to provide a higher diversity of these insects, as well as a higher number of insects at all (Büchs, 2003c). Specialists are less affected by landscape structures because they feed only on cruciferous crops during their whole life cycle (Jonsen and Fahrig, 2004).

Beside of the direct influence of landscape on oilseed rape visiting insects there is an indirect effect by its influence on natural enemies such as predators that were also investigated in this study. The landscape structure, location and regional characteristics of a field can have a detrimental effect on the abundance and diversity of spiders, *Staphylinidae*, *Tachyporus* and *Syrphidae* (Clough, 2006; Schmidt *et al*., 2005; Clough *et al*., 2007; Frank, 2006; Hausammann, 1996).

In conclusion, several factors are affecting the composition of insects and therefore the comparison of field trials from different years and which differ additionally in their site is extremely complicated. Therefore the influence of S-nutrition on insects can be superposed by other factors in the two years. Theses numerous factors had contrasting effects on specialist and generalist insect species of oilseed rape (Table 4.1). The occurrence and infestation of oilseed rape with insects was not only affected by S-nutrition and landscape structure but it could be affected by several other factors like weather, temperature, light, population dynamics of other insects, and plot size and other nutrients such as N (Westphal, 2004; Cannon, 1998). It is a special problem of field trials that not only one factor is changing like in pot trials but that always several factors are affected by the site and year of experimentation and the experimental conditions. Therefore it is very complicated to find consistently relationships when analysing one factor like it was done in this study and it is hard to compare different years of experimentation. The S-nutritional status is only one possible factor that is affecting the infestation of oilseed rape with different insects and already weak relationships between the S-nutritional status and the infestation of oilseed rape with insects are a good hint, that the S-nutritional status can affect the infestation of oilseed rape with pest insects.

Table 4.1: The whole number of different classified insect species from oilseed rape field in 2004 and 2005.

Insect species		Number of collected insects in oilseed rape 2004	Number of collected insects in oilseed rape 2005
Phytophagous pest	***Meligethes* spp.**	12240 [a]	4599 [a]
Oligophagous pest	***C. pallidactylus***	1428 [b]	816 [b]
	Ceutorhynchus obstrictus	2828 [b]	2380 [b]
	Dasineura brassicae	2524 [b]	1220 [b]
	***Phyllotreta* spp.**	748 [a]	Not found
	Brevicoryne brassicae	845 [a]	1066 [a]
Generalist pest	***Thysanoptera***	187 [a]	Not found
	Delia radicum	1439 [a]	1199 [a]
Polyphagous Pest	***Delia platura***	378 [a]	787 [a]
Generalist pest	***Delia florilega***	38 [a]	86 [a]
Polyphagous pest	***Scaptomyza flava***	9 [c]	22 [c]
Polyphagous predator	***Staphylinidae* family**	7088 [b]	7304 [b]
	***Tachyporus* spp.**	2900 [b]	1952 [b]
	Syrphidae	Not found	529 [a]

[a]: adults per 40 sweeps (collected in whole season by a sweep net); [b]: adults per m^2 (collected by emergence traps over the whole season); [c]: adults per plant (collected by suction trap in whole season).

4.2 *Relationship between the S-nutritional status of oilseed rape and oilseed rape visiting insects*

In the present work, insects were collected in S-fertilised and unfertilised plots from oilseed rape over two years of experimentation and over the whole vegetation period of oilseed rape. In both years the oilseed rape crops showed a clear response to S-fertilisation and the total S-content in the vegetative material at stem elongation increased with S-supply from 3.80 mg S g^{-1} to 12.6 mg S g^{-1} in 2004 and from 6.20 mg S g^{-1} to 13.2 mg S g^{-1} in 2005, respectively. For oilseed rape the critical value when plants show symptoms of S-deficiency is below 3.5 mg S g^{-1} in the vegetative material at stem elongation and S-contents below 5.5 mg S g^{-1} indicate to a situation of latent S-deficiency. Only if a value of 6.5 mg S g^{-1} is transgressed S is no longer a yield limiting factor (Schnug and Haneklaus, 1994; Haneklaus *et al*., 2007). As the above mentioned values represent medium values over the whole plots symptoms of S-deficiency were observed in the unfertilised plots in 2004 while in 2005 symptoms of S-deficiency were observed only on single plants of the plots which received no S-fertilisation. In general S-fertilisation increased the contents of primary and secondary S-containing compounds (Table 3.1) as already shown by Schnug *et al*. (1995). But S-application does not only affect the chemical composition of plants but also morphological and physiological characteristics such as colour, odour, and size and shape of the flowers and of the whole inflorescences (Schnug and Haneklaus, 1994; Haneklaus *et al*, 2005). Under conditions of S-deficiency the flowers of oilseed rape are smaller and the colour is changed from bright yellow to pale yellow which is very likely less attractive for flower visiting insects (Haneklaus and Schnug, 2005; Haneklaus *et al*., 2005). Moreover the odour is significantly changed with S-deficiency (Haneklaus *et al*., 2005) what will affect insects which choose their host plants preferably by the odour. The whole feature of the inflorescence of S-deficient flower is looser because of the smaller flowers and the appearance is more similar to a fading inflorescence. All these changes can directly affect the behaviour of visiting insects through changes in the attractiveness of the plant. Moreover, under conditions of S-deficiency the GSL-content of plants is reduced as well as other S-containing constituents which can result in a higher susceptibility to environmental stress (Bloem *et al*., 2005) and to generalist herbivores. On the other hand, the increasing synthesis of secondary plant metabolites or allelochemicals caused by S-fertilisation will probably increase the infestation of the crop with specialists which are attracted by secondary plant metabolites and their degradation products such as isothiocyanates from glucosinolate degradation (Mithen, 2001). Therefore the influence of S-fertilisation was different between different insect species of oilseed rape and even within the same genus. S-fertilisation increased the infestation of oilseed rape with *Phyllotreta* spp., *Ceutorhynchus pallidactylus*, *Ceutorhynchus obstrictus*, *Dasineura brassicae*, *Delia radicum* and *Brevicoryne brassicae*. The number of *Delia platura*, *Scaptomyza flava* and Thrips as well as most predators such as *Tachyporus* beetle, *Syrphidae* flies and spider was negatively correlated with S-fertilisation (Table 4.2).

Table 4.2: Influence of S-fertilisation on the occurrence of different insect species in oilseed rape at different growth stages (BBCH) and possible reasons for a changing infestation.

Insect species	Influence of S	BBCH	Supposed reason for changing infestation	References
***Meligethes* spp. (Adult)**	Negative	53,61, 62	GSL, propenyl isothiocyanates and GSH	Giamoustaris and Mithen, 1992; Mänd *et al*., 2004
	Positive	66, 71, 76	Cysteine, colour of flowers, S-containing volatile compounds	Giamoustaris and Mithen, 1992; Cook *et al*., 2006; Ruther and Thieman, 1997
***Meligethes* spp. (Larva)**	Negative	61,62, 63	GSL	Mänd *et al*., 2004
	Positive	66,69, 75	Cysteine	Matula and Zukalovä, 2001; Chang, 2004
***Ceutorhynchus pallidactylus* (Adult)**	Positive	61, 63, 65, 83, 66	GSL and their hydrolysis products, GSH, cysteine	Städler, 1992; Hothorn *et al*., 2006
***Ceutorhynchus pallidactylus* (Larva)**	Positive	61,63	GSL and their hydrolysis products, cysteine	Bartlet, 1996; Matula and Zukalovä, 2001
***Ceutorhynchus obstrictus* (Adult)**	Positive	61, 63, 66, 75, 86, 81	GSL, butenyl and pentenyl isothiocyanate, colour of flowers, cysteine	Mithen, 1992; Cook *et al*., 2006; Bartlet *et al*., 1997; Chang, 2004; Golberg and Meillon, 1948
***Ceutorhynchus obstrictus* (Larva)**	Positive	72, 73, 78, 83	Cysteine, GSH	Hothorn *et al*., 2006
***Dasineura brassicae*(Adult)**	Positive	65, 72, 76, 78, 86	GSL and their hydrolysis products, cysteine	Städler, 1992; Bartlet, 1996; Murchie *et al*., 1997
***Dasineura brassicae* (Larva)**	Positive	67, 73, 78, 83	Cysteine	Bartlet, 1996; Chang, 2004
***Phyllotreta* spp. (Adult)**	Positive	61, 63, 66, 72, 76, 83	Propenyl, benzyl, indolyl glucosinolates, GSH	Mithen, 1992; Nielsen, 1989; Hothorn *et al*., 2006
***Brevicoryne brassicae* (Adult)**	Positive	66, 78, 86, 75	GSL and their hydrolysis products; growth and development of plant	Yusuf and Collins, 1998
***Delia radicum* (Adult)**	Positive	63, 65, 75, 81	GSL and their hydrolysis products, cysteine	Marazzi, 2003; Jong and Städler, 1999; Ellis *et al*., 1999
***Delia florilega* (Adult)**	Positive	Whole season	Change in a combination of S-containing compounds rather than of a single compound	Hopkins *et al*., 1997, Baur *et al*.,1996
***Delia platura* (Adult)**	Negative	50, 66, 75	GSL and their hydrolysis products, GSH	Jong and Städler, 1999; Hopkins *et al*., 1997
***Scaptomyza flava* (Adult)**	Positive	61	Growth and development of plant tissues	No references
	Negative	64, 65, 79, 81	S- containing defence compounds	Mithen, 2001

The results of this work indicate to the possibility to decrease the infestation with *Meligethes* spp., *Delia platura*, *Scaptomyza flava* and Thrips by S-fertilisation. This result is in agreement with *Mänd et al.* (2004) who found that *Meligethes* beetle preferred to feed on unfertilised flowers of spring oilseed rape compared to those fertilised with S and micro fertilisers.

Oilseed rape is very susceptible to the infestation with *Meligethes* spp. at early spring. The female of *Meligethes* spp. deposit their eggs in the flower buds, mainly into buds 2-3 mm in length, causing serious damage to the flower buds (Hansen, 2003; Borg and Ekbom, 1996). This pest can cause a serious damage to crops and can reach sometime at very high infection of up to 100% as it was observed in oilseed rape in spring 2006 in Germany (Sauermann and Gronow, 2007). Both adults and larvae cause the drop down of buds and flowers resulting in podless stalks (Williams and Free, 1978; Frearson *et al*., 2005) and causing a reduced number of buds that are able to develop into pods what is reducing the yield potential significantly.

The seed loss caused by *Meligethes* spp. depends on number of beetles and on immigration time of *Meligethes* spp. into oilseed rape crops in relation to flower development (Williams and Free, 1978). An early attacks cause more serious damage than attacks that occur during later growth stages (Ferguson *et al*., 2006). Oilseed rape has a great capacity to compensate the damage caused by this pest at a low level of attack because oilseed rape produces a great number of small undeveloped buds especially on its side branches. These buds usually abort. If normally developed buds are damaged and lost, the buds on side branches grow out to form new flower and buds (Hansen, 2004). Hiiesaar *et al*. (2003) found no significant decrease in yield when 40% of buds were removed from the plant. However, the yield is severely reduced at higher levels of attack (Hansen, 2003). Use of insecticide against this beetle is considered economically sound when at least 25% of pods are infected (Hiiesaar *et al*., 2003). However, the resistance of *Meligethes* spp. to pyrethroid insecticides in Europe (see introduction) could decrease the efficiency of insecticide application (Veromann *et al*., 2006). Therefore, it is important to find alternative strategies to reduce the damage caused by this pest. This study showed that application of S-fertilisers decreased damage of *Meligethes* spp. by decreasing the number of adults and infection rate of buds at early flowering (Fig. 3.6; 3.7) and by improving the ability of plant to compensate the damage caused by this pest. If the plants are in a good condition before the attack of *Meligethes* spp., they will be able to compensate more easily (Hansen, 2003). Therefore, the nutritional status is a significant factor affecting *Meligethes* spp. attacks (Hansen, 2003). Application of S-fertilisation with insecticide together decreased the infestation level with *Meligethes* spp more than application of S-fertilisation alone. At BBCH 61, S-application decreased the infection rate of oilseed rape with *Meligethes* spp. by 11% (from 36.4 to 32.3) in 2004 and the infection rate decreased by 15% (19.4 to 16.4) in 2005 when insecticide was sprayed additionally. At BBCH 63, the infection rate by *Meligethes* spp. was decreased by 15% (from 53.3 to 45.5) when S-fertiliser was applied without insecticide and reduction by 25% (from 41.5 to 31.2) was achieved when S-fertilisers were used together with insecticides.

Delia platura is an important pest in agricultural fields. The maggots attack the roots of various Fabaceae, tobacco, cereals, and tubers seedlings, which can then be attacked by stem rot organisms. Crop yield is reduced when infestation levels are high (Gouinguene and Städler, 2006). *Delia platura* has four generations per year and the duration of development is much shorter than for *Delia radicum*. This pest is attracted by organic substances and the first larval stages are able to survive by feeding from organic substances until emergence of plants (Büchs and Prescher, 2006). Oilseed rape is very susceptible to the infestation with *Delia platura* at early spring (Jong and Städler, 1999). Larvae of *Delia platura* cause the highest level of damage in early spring when they feed on the roots. The number of adults of *Delia platura*, as well as the damage to the oilseed rape crop decreased with S-fertilisation (Fig. 3.40).

Scaptomyza flava and Thrips as polyphagous insects are no important pests in oilseed rape cropping. Adults of Thrips feed inside the developing flower bud and newly expending leaves. *Scaptomyza flava* is one species of leaf miner flies, which is regarded as a pest of minor relevance. Most of the damage caused by the leaf mining fly is attributed to the larvae that mainly occur in leaves and stems of plants and bore into the tissues of plant leaves as well. But it does not affect the growth and yield of oilseed rape very much. In the present study, *Scaptomyza flava* (Fig. 3.40) and Thrips were negatively affected by S-application.

Besides that on *Meligethes* spp., a positive effects of S-fertilisation was also found on infestation of oilseed rape with different species of *Ceutorhynchus* genus like adults of *Ceutorhynchus pallidactylus* and *Ceutorhynchus obstrictus* in addition to larvae of *Ceutorhynchus pallidactylus* and *C. napi* during early spring, flowering and pod ripening (Fig. 3.21). Also, S-fertilisation had a positive effect on oviposition behaviour of stem mining weevils (*Ceutorhynchus pallidactylus* and *Ceutorhynchus napi*) at early flowering and for *Ceutorhynchus obstrictus* at flowering which are the most susceptible vegetation periods for adults during laid egg.

Dasineura brassicae started to colonise oilseed rape crops at main flowering, when female adults deposited their eggs in pods (Ferguson *et al*., 1995). Therefore the influence of S-fertilisation on the infestation level is most important at flowering. This study illustrated that the infestation of oilseed rape with *Dasineura brassicae* increased with S-application during flowering, pod development and ripening (Fig. 3.30).

The results of the present work show that *Delia radicum* preferred and developed better on plants which were better supplied with S (Fig. 3.40). This result is in accordance with Marazzi (2003) who indicated that the S-supply through its effect on the GSL-content and by this the isothiocyanate content can affect the host-plant acceptance and oviposition behaviour of cabbage root flies.

Only a slightly effect of S-fertilisation on the occurrence of cabbage aphids was found in this study. This result is in accordance with Yusuf and Collins (1998) who found a positive correlation between the GSL level influenced by S-application and the feeding performance of cabbage aphids.

It can be concluded from the present results that the application of 150 kg S ha^{-1} increased the infestation level with *C. pallidactylus*, *C. napi, Ceutorhynchus obstrictus*, *Dasineura brassicae*, *Delia radicum* and *Brevicoryne brassicae* at the relevant growth stages of oilseed rape when the crop was most susceptible to these pests.

Generally, S-fertilisation increased the total S-content, cysteine, GSH, and GSL-content in oilseed rape plant tissues and these higher contents can either positively or negatively affect the composition of oilseed rape visiting insect (Table 3.1).

Possible mechanisms by which these S-containing compounds can affect insects are discussed in the following chapter.

This study showed also that *Ceutorhynchus pallidactylus* was the most serious pest in 2004 while *Meligethes* spp. caused a higher damage in 2005. In 2004 46% of stems were infected by larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* and additionalles 28% of flower buds were destroyed by adults and larvae of *Meligethes* spp.. In 2005, 16.5% of the flower buds were destroyed by *Meligethes* spp., while the infection rate of stems with *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* was low with a percentage of 3.95%. The pest which mainly affect the pods like *Ceutorhynchus obstrictus* and *Dasineura brassicae* did not differ very much inboth years of experimentation. The infestation rate with *Ceutorhynchus obstrictus* and *Dasineura brassicae* was 8.9% and 9.4% respectively in 2004 and 10.1% and 9.2% respectively in 2005.

Influence of GSL on oilseed rape visiting insects

The GSL-content of leaves as well as seeds of oilseed rape is highly influenced by the S-nutritional status of the crops as shown in several studies (Schnug, 1988; Salac, 2005). The influence of GSLs on different insects species was variable (Table 4.2), because GSLs and their degradation products can either be a deterrent to generalist herbivores or an attractant and stimulant to specialist herbivores (Mithen, 1992; 2001).

Adults of *Meligethes* spp. were positively affected by S-fertilisation during flowering, pod development and ripening (Fig. 3.7). This increase can be explained by the influence of GSLs, S-containing volatile compounds and isothiocyanates that play a major role in host plant location, oviposition and which act as feeding stimulants for *Meligethes* spp. adults during flowering, as it was reported by Cook *et al*. (2006), Giamoustaris and Mithen (1992), and Ruther and Thieman (1997). Also, during flowering the beetles can avoid the breakdown of GSLs because they can feed directly on the pollen without having to damage any tissues.

GSLs and their degradation products are important plant defences compounds in crucifers such as oilseed rape. The hydrolysis of GSLs is related to the endogenous enzyme myrosinase that is stored separately from GSLs in plant tissues, and which does not react with GSLs until the tissue is damaged. The enzyme is thought to act as a thioglucosidase to produce an unstable aglucone, which then can form several products (thiocyanates, nitrile, oxazolodine-thione) dependent on the nature of the GSL side chains and other factors such as pH, the presence of ferrous ions and the ascorbic acid concentration (Mithen, 1992). Alkenyl-GSLs form stable isothiocyanates following a loose rearrangement of the aglycone. Only certain GSLs (for example, 3-butenyl GSL) release isothiocyanates when they are metabolised. The GSL-myrosinase system is affected by several factors such as S-fertilisation, balance between N and S, abiotic stress and biotic stress, for example, by insects. There are different theories regarding the potential role of the GSL-myrosinase system in the plant (Wretblad, 2002). GSLs as a defence system seems to be mainly effective against generalist insects, which are not adapted to GSL-containing hosts while specialist insects utilise GSL or their degradation products to locate their host, and to stimulate feeding and oviposition. Several studies have demonstrated increasing plant damage by specialists with increasing GSL-contents (Mitchell, 1996; Lambdon *et al*., 1999). There are degradation products from GSLs that are toxic compounds to generalist insects while they are beneficial for specialists such as *Brevicoryne brassicae*, *Plutella xylostella*, *Phyllotreta* spp., *Ceutorhynchus obstrictus*, and *Dasineura brassicae* on oilseed rape. The host location is mediated by a combination of visual and olfactory parameters in phytophagous specialist insects while oligophagous specialists orientate on secondary plant compounds. A high GSL-content may protect plants from generalist insects but for specialists different results were found (Hopking *et al*., 1997).

Higher GSL-contents, and thus higher concentrations of degradation products in S-fertilised plants, can be the reason for the negative influence of S-application on *Delia platura*, *Scaptomyza flava* and Thrips as polyphagous insect as well as for adults and larvae of *Meligethes* spp. as phytophagous insects. GSLs have a significant allelopathic potential (Selmar, 2005) which thought to be involved in the plant defense against generalist. Also when the insects feed on the plant tissue, GSL breakdown products and other defence compounds are build (Jong and Städler, 1999) and the higher concentrations in S-fertilised plants seem to have a repellent effect for larvae of *Meligethes* spp. as well as for adults of *Delia platura, Scaptomyza flava* and Thrips. The negative correlation was found between the GSL-content and the number of adults and larvae of *Meligethes* spp. at early flowering (Table 4.3).

During feeding tissue is damaged and GSLs came in contact with the myrosinase which is degrading the intact GSLs (Fig. 4.1).

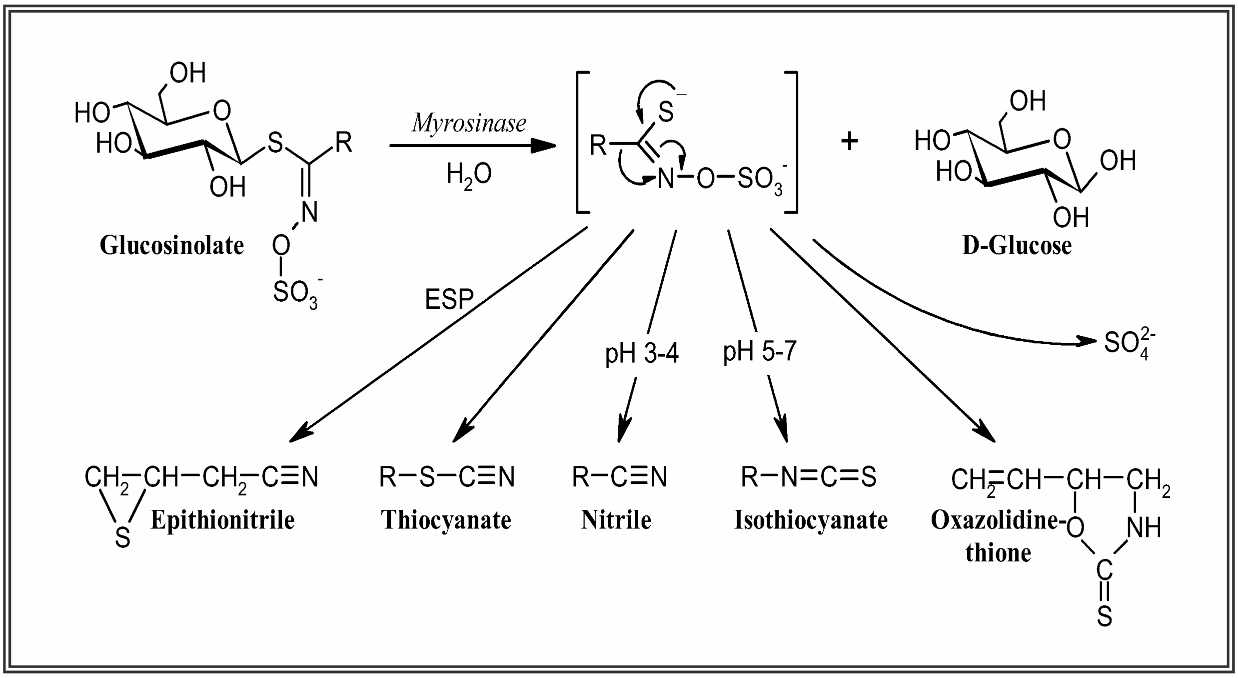


Fig. 4.1: Hydrolysis of glucosinolates by myrosinase and possible reaction products (Wretblad, 2002).

Higher contents of breakdown products seem to have an attractive effect to *Dasineura brassicae, Ceutorhynchus obstrictus, Ceutorhynchus pallidactylus, Phyllotreta* spp. and *Brevicoryne brassicae* for feeding, host location and egg deposition. For example, allyl-isothiocyanate serves as a cues or stimulant which helps *Dasineura brassicae* in host location during oviposition as reported by Städler (1992) and allyl-glucosinolates are considered to be feed stimulating for *Brevicoryne brassicae*. Isothiocyanates and volatile S-containing compounds are very important for oviposition as well as larval performance and feeding behaviour of *Delia radicum* too (Jong and Städler, 1999; Ellis *et al*., 1999). Additionally, also the larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* are attracted by the breakdown products of GSLs which are build when the tissue is damaged (Bartlet, 1996). Moreover also adults of the *Ceutorhynchus obstrictus* are reported to be attracted by isothiocyanates and volatiles such as nitriles, goitrin, and probably indole which act as feeding stimulant and cue for host location (Mithen, 1992; Cook *et al*., 2006; Bartlet *et al*., 1997) while indolyl-glucosinolates are considered to act as feeding stimulant for *Phyllotreta* spp. (Mithen, 1992; Nielsen, 1989).

Influence of cysteine and GSH on oilseed rape visiting insects

The positive effects of S-fertilisation on the infestation of oilseed rape with different pest species are probably caused by the increase in the cysteine-content. After sulphate assimilation cysteine is the first stable organic S-containing compound in plants and is the precursor of all other S-containing metabolites like GSH and GSL. The cysteine-content regulates the sulphate uptake and assimilation in plants and is an important amino acid in the biosynthesis of proteins. Higher levels of free amino acids have an additional function that is related with neural transmission, detoxification, and synthesis of phospholipids, energy production, morphogenetic processes that have important biological roles (Chang, 2004). There are some hints that the cysteine-content of the plant is importance for its food value to larvae. For example Chang (2004) could show that the number of laid eggs by *Ceratitis capitata* was significantly lower when they fed on a diet lacking in cysteine. The deletion of cysteine in the diet of *Ceratitis capitata* reduced the lifetime, total oviposition and eggs viability of this insect (Chang, 2004). Cysteine is an essential part in nutrition and feeding of larvae, and also for the development of eggs, because it is a component of full-value proteins and plays an important part in nutrition, especially if it is not replaced by methionine in certain circumstances (Matula and Zukalovä, 2001). Moreover, cysteine acts as a feeding stimulants and is important for the development of some insects such as Mealybug

(*Phenacoccus herreni*) (Calatayud *et al*., 2002), *Aedes aegypti* (Chang, 2004) and *Oryzaephilus*. Chang (2004) found that *Oryzaephilus* insects can develop well without leucine, lysine, phenylalanine, but require cysteine and glycine in their food source. In present work, an increasing reproduction success of *Ceutorhynchus obstrictus* was observed with increasing cysteine content in the vegetative plant material (see chapter 3.5). This result was in agreement with Golberg and Meillon (1948) who showed that cysteine is important during pupation and lower level of cysteine resulted in a higher proportion of *Aedes aegypti* adults which failed to emerge. For all of these reasons the S-fertilised plants represent a much better food service which support egg laying of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* and is a richer food resource for the development of larvae of *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus*, *Ceutorhynchus obstrictus* and *Dasineura brassicae*. Higher numbers of emerging adults of *Ceutorhynchus obstrictus* and *Staphylinidae* in relation to a higher S-supply can be also related to the function of cysteine for larva development. A positive correlation was observed between the cysteine-content and the occurrence of larvae of *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus*, *Ceutorhynchus obstrictus* and *Dasineura brassicae* as well as between the cysteine-content and laid eggs by *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* (Table 4.3). This positive correlation confirmed that no indirect effect of S-fertilisation but the increase of S-containing compounds caused the increased the higher infestation level.

GSH belongs to the S-containing compounds that are clearly related to the stress response of the plant. The various roles of GSH for plant development (Wachter and Rausch, 2005), stress tolerance against abiotic and biotic stress (Schnug *et al*., 2005) highlight its central role as an important S-metabolite with multiple function. These various functions are reflected in different response of oilseed rape visiting insects as shown in table 4.2 and 4.3.

The GSH is part of the anti-oxidative system of plant cells and is involved in the detoxification of xenobiotics, it serves as a major defence component against a wide range of biotic stress factors (Hothorn *et at*., 2006) and acts as a source for the metabolism of other S-containing compounds, which are important in S induced resistance (Bloem *et al*., 2004). After an insects attack some of these defence compounds such as GSH increase and especially GSH may act as systemic messenger carrying information concerning the attack to non-infested tissues.

A positive relationship was found between the GSH-content and the infestation of oilseed rape with adults of *Ceutorhynchus pallidactylus* (Table 4.3). A negative relationship was found for *Meligethes* spp. and *Delia platura* and the GSH-content and this can probably explain the negative influence of S-fertilisation on these insects in early spring in 2004.

Generally the GSH-content of the plant is changing very rapidly therefore correlation are hard to find especially as the plant material was only analysed at stem elongation and not with every insect sampling (Bloem *et al*., 2007).

The results showed that the different plant constituents like cysteine, GSH and GSLs changed with S-fertilisation and this change in S-containing compounds seems to have an effect on generalist and specialist insects of cruciferous crops which seem to be promoted while generalist insects were deterred by higher contents of S-containing compounds.

Table 4.3: The Pearson correlation between S-compounds in leaves of oilseed rape and the occurrence of oilseed rape visiting insects in 2004.

Parameter	S-content	GSL	GSH	Cysteine
Egg-laid by *C. napi* and *C. pallidactylus*	0.57 (*)	0.11	0.37	0.51 (*)
Larvae of *C. napi* and *C. pallidactylus*	0.34	0.07	0.49	0.56 (*)
Larvae of *Meligethes* spp.	-0.43	-0.43	0.37	0.14
Larvae of *Ceutorhynchus obstrictus*	0.24	0.39	0.38	0.52 (*)
Larvae of *Dasineura brassicae*	0.59 (*)	0.51 (*)	0.26	0.71 (**)
Adults of *Delia platura*	- 0.91	0.22	-0.69(**)	-0.18
Thrips	- 0.40	-0.55(*)	-0.00	-0.33
Adults of *Scaptomyza flava*	0.00	-0.07	0.45	0.24
Adults of *Delia radicum*	0.59	0.14	0.15	0.45
Adults of *Phyllotreta* spp.	0.13	-0.41	0.49	0.00
Adults of *C. pallidactylus*	0.05	-0.05	0.53 (*)	0.14
Adults of *Brevicoryne brassicae*	0.24	0.40	-0.20	0.42
Adults of *Dasineura brassicae*	0.59 (*)	0.13	0.18	0.46
Adults of *Meligethes* spp.	- 0.13	-0.09	-0.45	-0.21

n= 16; ** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

Classification of the influence of S-nutrition on oilseed rape visiting insects by hierarchical cluster analysis

It was clear from this work that the response of different insect species to S-fertilisation was different. Some of the insect pests were positively affected by S-fertilisation while other species were reduced, probably because of defence compounds which are enhanced by S-fertilisation. The cluster analysis has the objective to sort and classify insects into groups or clusters, so that the degree of association is strong between members of the same cluster and weak between members of different clusters. There are a number of different algorithms and methods for grouping objects of similar kind into respective categories (Everitt, 1993). The insects were classified into groups based on patterns of correlation among each other. A hierarchical cluster analysis was run, applying Pearson correlation as the similarity measure. In this case cluster analysis allows classifying the pests into subgroups that have similar response patterns to S-application. A hierarchical cluster analysis based on the response of oilseed rape visiting insects to S-fertilisation was conducted, and the insects were classified into two separated groups (A and B). The first group (A), which included adults of polyphagous insects (*Scaptomyza flava*, *Thrips* and *Delia platura*), was negatively affected by S-fertilisation. The second group is comprised of oligophagous insects (*Phyllotreta* spp., *Ceutorhynchus obstrictus*, *Ceutorhynchus pallidactylus*) that appeared to be positively affected by S-fertilisation (Fig. 4.2).

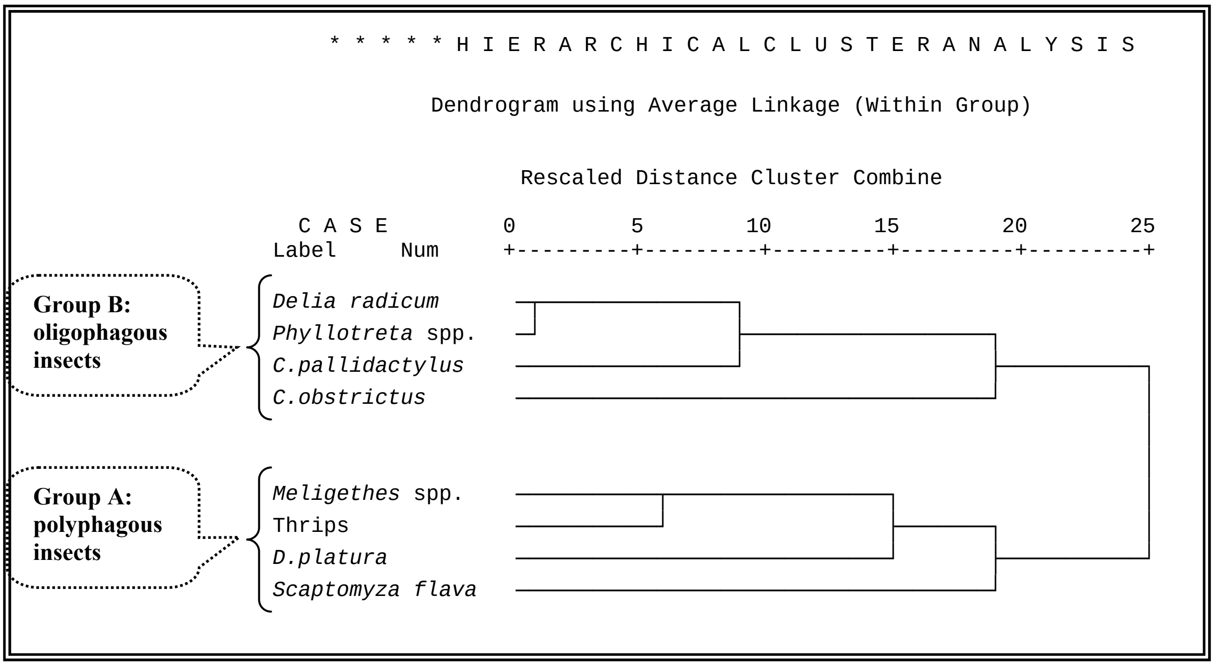


Fig. 4.2: Hierarchical cluster analysis of the response of oilseed rape visiting insects to S-fertilisation at early flowering in 2004 (group A: polyphagous insects, group B: oligophagous insects) (Insects were collected by sweep net).

At early flowering S-fertilisation decreased the infestation of flower buds with adults and larvae of *Meligethes* spp.. During this stage, *Meligethes* spp., as a phytophagous insect, appeared to act in a similar way to the other polyphagous insects from group (A) because they were deterred by a higher content of S-containing metabolites. Most likely the first group was negatively affected by a higher GSL-content and thus a higher content of degradation products through S-fertilisation. These compounds act as a deterrent or repellent for polyphagous insects and on the other hand as attractive cues for feeding, oviposition and host plant location for specialists. Adaptation to defence compounds of plants differ between insect species. Specialist herbivores restrict their counter defensive measures to the small range of defensive tactics of the plants on which they are specialised, while generalists have to invest in broad detoxification strategies (Agrawal and Kurashige, 2003). Furthermore *Brevicoryne brassicae,* and *Phyllotreta* spp. as specialists for cruciferous crops, have co-evolved with the defence compounds of their host plants (Pontoppidan *et al*., 2001) and possess their own myrosinase activity with which they can detoxify GSLs.

The same groups of insects were classified using cluster analysis during full flowering again (Fig. 4.3). Different results were found for *Meligethes* spp. and *Delia radicum* compared to the results from early flowering. The first group (A) reflects the polyphagous insects (*Scaptomyza flava*, *Thrips* and *Delia platura*) which were negatively affected by S-fertilisation because they have no adaptation or detoxification mechanism against plant defence compounds that increase with S-application. Group B includes *Phyllotreta* spp., *Ceutorhynchus obstrictus*, *Ceutorhynchus pallidactylus, Dasineura brassica*e and *Brevicoryne brassicae* as well as *Meligethes* spp. and *Delia radicum*. The *Phyllotreta* spp., *Ceutorhynchus obstrictus*, *Ceutorhynchus pallidactylus, Dasineura brassicae* and *Brevicoryne brassicae* are oligophagous specialists for cruciferous crops. A positive correlation was found with S-containing compounds as mentioned earlier.

Adults of *Meligethes* spp. were attracted by S-fertilised plants like the cruciferous specialists. A possible explanation for this positive attraction is the fact that adults at this time feed on pollen without the need to damage the plant tissue. Therefore the enzyme myrosinase will not come in contact to the GSLs and no degradation is activated. Additionally the bright yellow flowers of S-fertilised plants will be an attractant for *Meligethes* spp.. Adults of *Delia radicum* are attracted by another mechanism: here the isothiocyanates and volatile compounds act as an oviposition and feeding stimulant (Jong and Städler, 1999).

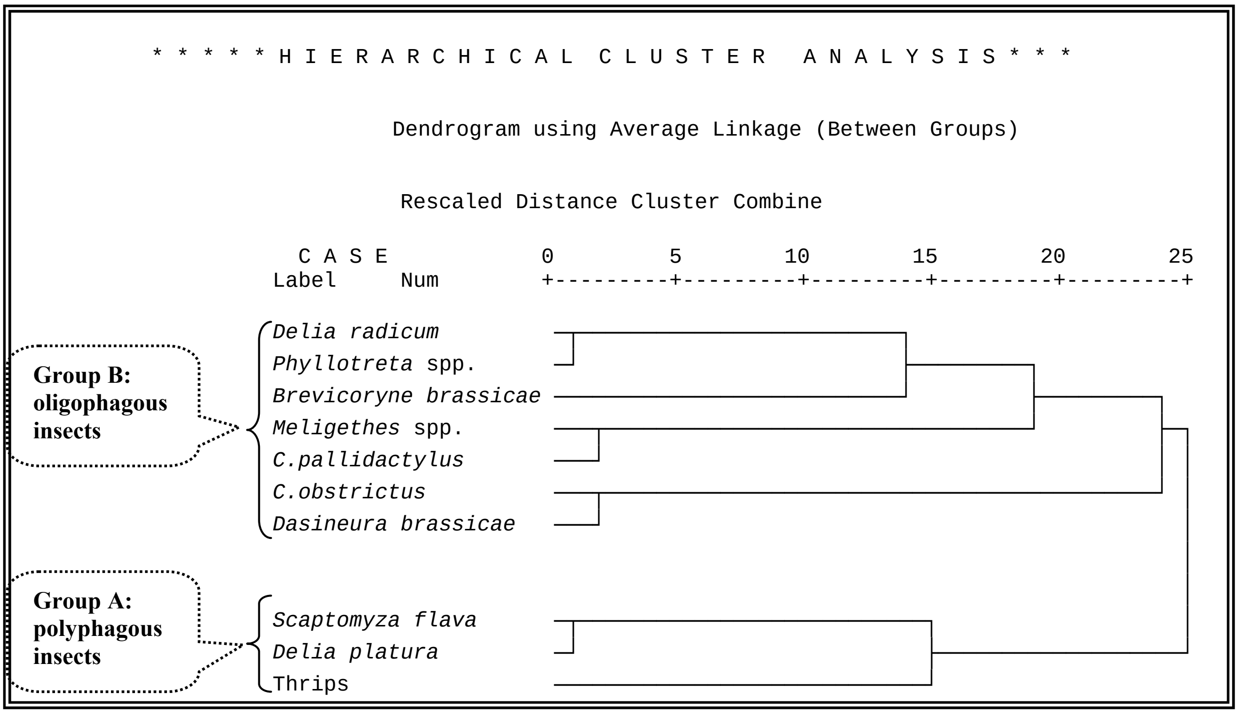


Fig. 4.3: Hierarchical cluster analysis of the response of oilseed rape visiting insects to S-fertilisation at full flowering (group A: polyphagous insects, group B: oligophagous insects) (*Phyllotreta* spp, *Delia radium* and *Scaptomyza flava* were collected by suction trap, while the other insects were collected by sweep net).

The attraction of different insect species to S-fertilised plots of oilseed rape was similar during flowering, pod development and ripening. The only exception was *Delia radicum* (Fig. 4.4). Adults of *Delia radicum* showed a similar behaviour to the polyphagous insects in group A at pod ripening.

The results of the hierarchical cluster analysis clearly reveal a differentiation between specialist (oligophagous insects) and generalist insects (polyphagous insects) with respect to the influence of S-fertilisation. Specialist insects feed on a small number of plant species which are related chemically and taxonomically. Therefore they use their hosts more efficiently than generalist species, which feed on a wide range of plant species. As a result, specialist insects were adapted to the variation in quantity or quality of defence compounds of their host plant.

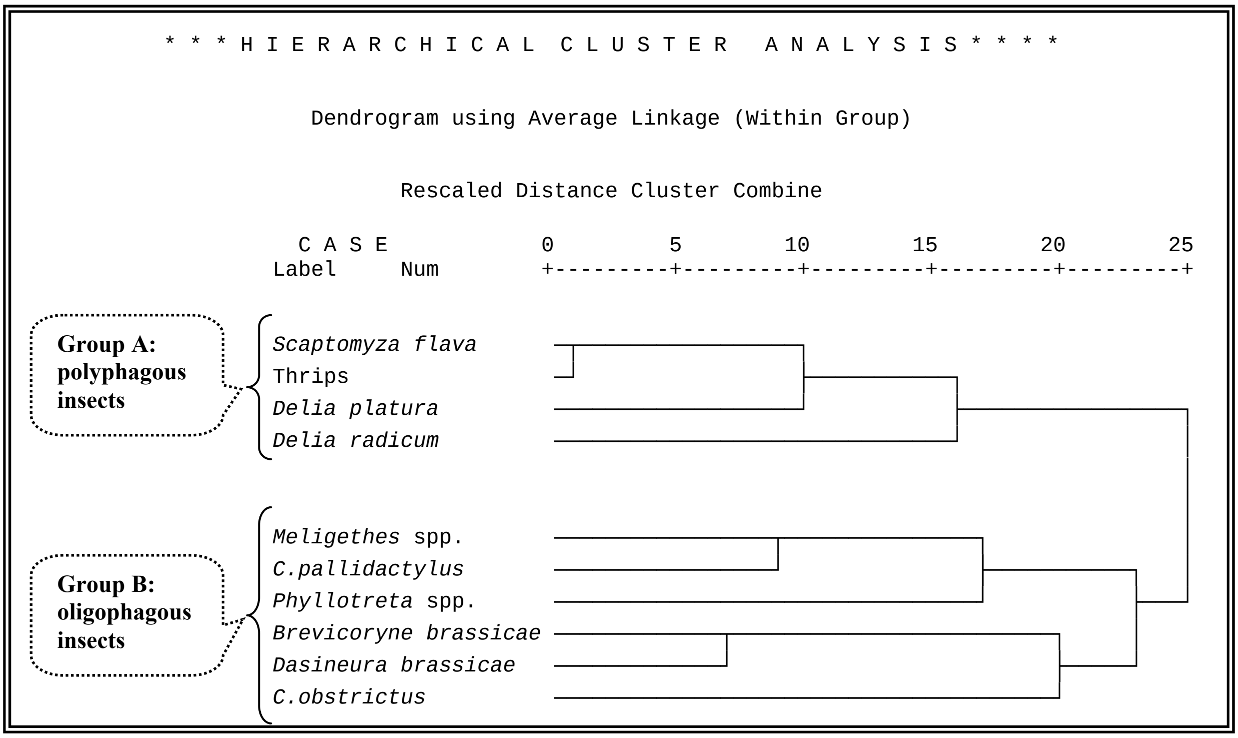


Fig. 4.4: Hierarchical cluster analysis of the response of oilseed rape visiting insects to S-fertilisation at pod ripening (group A: polyphagous insects; group B: oligophagous insects) (*Meligethes* spp., *Brevicoryne brassicae*, *C. obstrictus* were caught by sweep net; *Dasineura brassicae*, Thrips, *Scaptomyza flava* were collected by suction trap while the other insects were collected by emergence traps).

4.3 *Relationship between S-fertilisation and beneficial insects of oilseed rape*

It was shown in the last chapter that the S-supply had a strong influence on different oilseed rape visiting insects. These changes will also affect the population dynamic of predators which play an important role in controlling pests and reduce their potential damage (Steinbrecher, 2004). Therefore, S-fertilisation may have an indirect effect on predators. For example, parasitoids and predators can use S-containing volatile compounds to find the location of their preys (see introduction) (Hilker and Meiners, 2002; Gatehouse, 2002; Birkett *et al*., 2000; Bartlet, 1996, Venzon *et al*., 1999). Also GSLs and their degradation products are important in the interaction of plants with insects (Steinbrecher, 2004) and interaction between herbivores insects and their natural enemies (Harvey *et al*., 2003). On the other hand S-fertilisation increased S-containing compounds such as GSL which can act as feeding deterrents, change the development and physiology of herbivores, reduce growth rates, give adults with smaller size and increase mortality (Giamoustaris and Mithen, 1992). These compounds can be sequestered in the body tissue of herbivores and affect natural enemies indirectly by delayed development, reduced hatching rates and low performance (Stamp and

Bowers**,** 2000). Specialist herbivores have developed mechanisms to detoxify the plant defence compounds (see introduction). Furthermore, they can sequester these toxic compounds and use them against their predators (Pasteels *et al.*,1988) and this made them less preferred preys. This study showed that the numbers of *Staphylinidae*, *Tachyporus* spp., *Syrphidae* and spider were decreased by S-fertilisation (Table 4.4). The reason for this could be that their prey feed on S-fertilised plants containing high level of plant defence compounds compared with those feeding on unfertilised plants and these compounds result in adverse effects on predators. This result is in agreement with Van der Meijden and Klinkhamer (2000), who found that an increase of plant defence compounds had a negative effect on natural enemies of generalists and specialists. Only monophagous predators can adapt to defence compounds in their herbivore preys (Van der Meijden and Klinkhamer, 2000).

Table 4.4: Response of beneficial insects in relation to S-fertilisation at main growth stages of oilseed rape.

Insect species	**Relative changes in the occurrence of beneficial insects (adults and larvae) with S-fertilisation in relation to control (%)**			
	Early bud stage	Flowering	Pod development	Pod ripening
***Staphylinidae* (Adult)**	0 *	0	-47	-21.5
***Staphylinidae* (Larvae)**	0 *	0	-24.3	-6
***Tachyporus* (Adult)**	0	0	-68	-47
***Tachyporus* (Larva)**	0	0	-24	-12
***Syrphidae* (Adult)**	-100	-68	-55	-44
Spider	+500	+35	-2	-8

* : Adults of *Staphylinidae* as well as adults and larvae of *Tachyporus* spp. appeared only after flowering and no individuals were collected at early growth stages. (insects were collected by sweep net at early bud stage and flowering while they monitored by emergence traps at pod development and ripening stages).

Rove beetles (Coleoptera: *Staphylinidae*) are polyphagous predators of different oilseed rape pest species. Adults and larvae of this predators negatively affected with S-supply (Fig. 3.45, 3.46) (A). This decrease is probably caused by the positive effect of S-fertilisation on some of the specialist insects which can use S-containing compound to deter their enemies like *Staphylinidae*.

This study indicated that adults of *Tachyporus* were negatively affected by S-fertilisation (Fig.3.47). Also *Tachyporus* as a polyphagous predator feed on different oilseed rape pest species, but they have preferences in their feeding choice. For example, it was reported that *Tachyporus hypnorum* significantly preferred to feed on larvae of *Meligethes aeneus* in comparison to larvae of *Dasineura brassicae* (Schlein and Büchs, 2004). The appearance of larvae of *Tachyporus* coincided with the main period were full grown larvae of *Meligethes* spp. dropped to the soil. On the other hand this study showed positive response of *Meligethes* spp. to S-fertilised plants and they can use the defence system of the plant (e.g. sequestration of GSLs) as a protection against predators (Müller *et al*., 2003). Since the GSL-myrosinase system acts as a defence mechanism against generalist herbivores, it could be also act against *Tachyporus* as polyphagous predator that has no mechanism to deal with these toxic defence compounds (Aliabadi *et al*., 2004).

The same can be of relevance for adults of the hoverfly (*Syrphidae*), which population is also negatively affected by S-fertilisation during inflorescence emergence and flowering (Fig. 4.53). This predator was not affect by S-supply during pod development and ripening stages in spite of changed number of their preys (*Brevicoryne brassicae*) (Fig.3.54). The overall effectiveness of aphidophagous *Syrphidae* larvae as regulators of aphid infestations on crops was reduced in 2005 because adults appear to late when the aphid population has already reached critical levels (Büchs, 2003a).

A relationship between predators and the S-nutritional status of oilseed rape could not be expected as predators do not feed on plant material but on insects. But as the S-nutritional status had a significant effect on some of the prey insects of the predators indirect effects of S-nutrition were expected and such indirect relationships were observed for *Staphylinidae*.

The population of *Brevicoryne brassicae*, which is one important prey of spider, was not the only factor which was affecting the spider population because there are several other factors such as season and location which are important for the composition of their preys (Büchs, 2003a). However, the peak occurrence of *Brevicoryne brassicae* and spiders coincided at full pod development (BBCH 76) (Fig. 3.55) but the population of *Brevicoryne brassicae* increased with S-fertilisation while the population of spiders decreased probably as a result of increasing S-defence compounds in *Brevicoryne brassicae* with S-fertilisation. Steinbrecher (2004) found that the increase of defence compounds in the prey tissue increased mortality of predators. The results showed that the composition of oilseed rape visiting insects is not only directly influenced by the S-nutritional status of the crop but also indirectly with view to the predator insects which population depend on the occurrence of pest organisms.

4.4 *Relationship between N-fertilisation and infestation of oilseed rape with different pests and beneficial insects*

The results showed that higher doses of N-application (200 compared to 100 kg ha^{-1}) significantly increased the density and population of adults of *Meligethes* spp., *Ceutorhynchus pallidactylus, Ceutorhynchus obstrictus, Dasineura brassicae, Delia radicum, Delia platura, Brevicoryne brassicae, Staphylinidae* family and *Tachyporus* genus. This positive effect of N-fertilisation on the population of different insects was not only observed on adults of oilseed rape visiting insects but also on their larvae. The larvae of *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus, Ceutorhynchus obstrictus, Dasineura brassicae and Tachyporus* genus preferred to feed on plants that received a higher dose of N and the number of eggs of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* in stems was higher in plants which received 200 kg N ha^{-1}. N is an important plant nutrient which increased the growth of the plant, and the protein content which is considered to be important for the development of eggs, larvae and pupates of insects (Bruyn *et al*., 2002). N-fertilisation generally reduces physical plant defences (such as trichomes and spine) and also chemical plant defence compounds which is beneficial for the growth and development of adults and larvae (Chen and Welter, 2005). Moreover N-fertilisation increases the size of flowers, which increases the attractiveness for adult insects and results in a higher amount of eggs on the petals as indicated by Jansson (2003). N-fertilisation also increases the amount of some essential amino acids which are important for hatching eggs and the growth and development of larvae (Chang *et al*., 2004).

This study also indicated a relationship between the N- and S-nutritional status of the crop on oilseed rape visiting insects as the highest number of *Meligethes* spp., *Ceutorhynchus pallidactylus, Dasineura brassicae, Ceutorhynchus obstrictus* and *Brevicoryne brassice* were collected from plants that were fertilised with S and received a high dose of N. The highest infestation with adults of *Dasineura brassicae* (Fig. 4.5) was observed in plots that received 150 kg S ha^{-1} and 200 kg N ha^{-1}. *Scaptomyza flava* showed a different trend: the highest number of these insects was captured in plots that received no S-fertilisation, but N at the higher dose of 200 kg N ha^{-1} (Fig. 4.6). N and S are closely related to each other in the plant metabolism as both elements are used in protein biosynthesis, and the balance between N and S regulates the synthesis of proteins and the accumulation of GSLs (Fismes *et al*., 2000). For example, the N-nutrition can increase or decrease the GSL-content of rapeseed, depending on S-supply. Higher N-doses decrease the GSL-content in the absence of S, but increased the GSL-content when S is available. When S is a limiting factor, most S is incorporated into primary products (proteins), and less S is available for the synthesis of secondary S-containing compounds like GSLs. An increasing N-supply increases also the demand for S for the primary metabolism, and thus suppresses the synthesis of GSLs even more (Schnug, 1988). Less GSLs can cause higher infestation of oilseed rape with generalist insects that have a limited adaptation to S-containing defence compounds. However, under a sufficient S-supply, an increasing N-supply will enhance the synthesis of amino acids, which are the precursors for GSL biosynthesis, and the population of specialists like *Dasineura brassicae* will also increase as it is shown in Fig. 4.5. The combination of high N- and high S-fertilisation resulted in the highest population of adults of *Dasineura brassicae*.

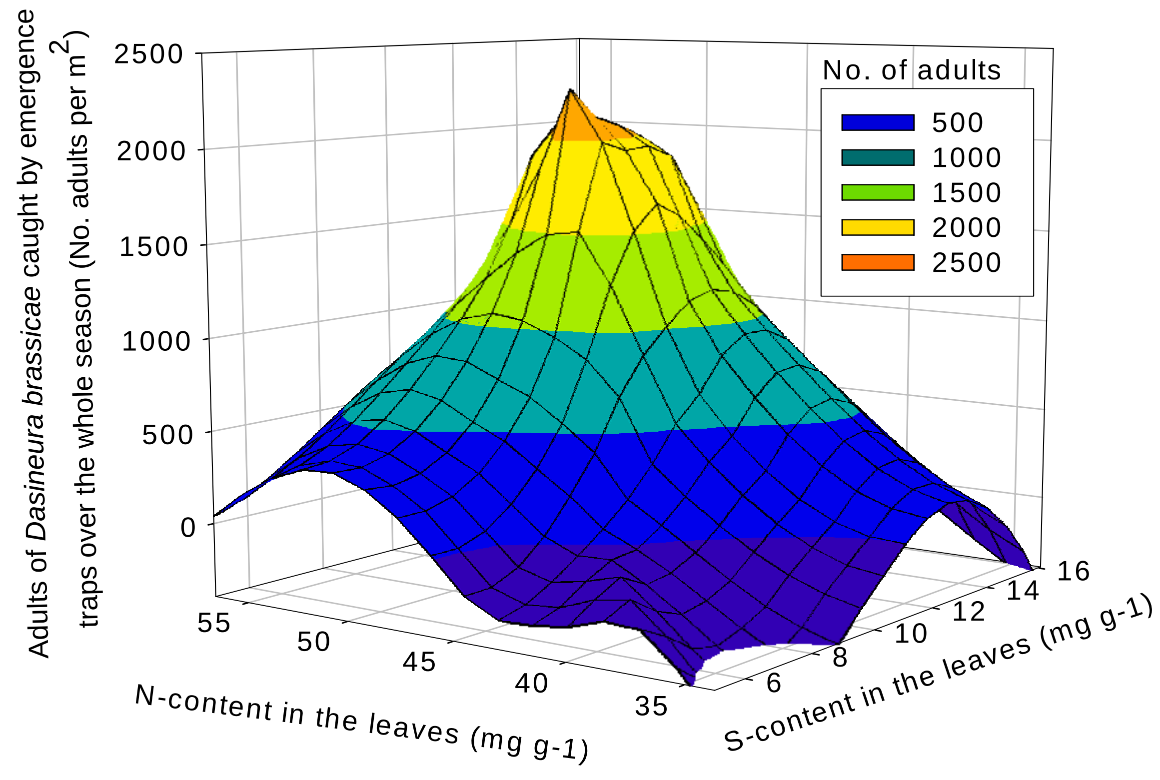


Fig. 4.5: Infestation of oilseed rape with adults of *Dasineura brassicae* collected over the whole season by emergence traps in relation to the S- and N- nutrition of the crop at stem elongation in 2005.

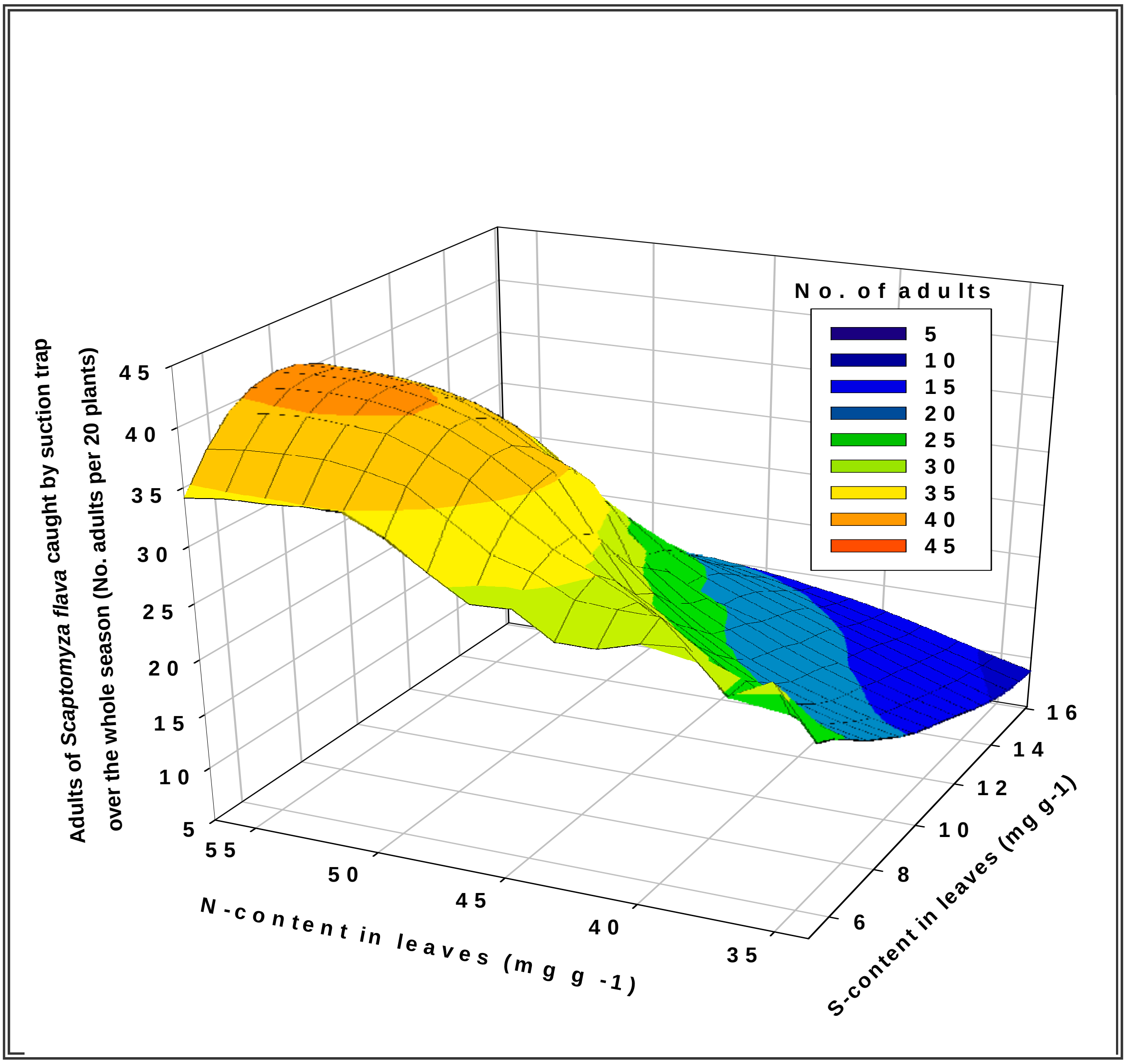


Fig. 4.6: Infestation of oilseed rape with adults of *Scaptomyza flava* collected over the whole season by suction trap in relation to the S- and N- nutrition of the crop at stem elongation in 2005.

The present study illustrated that S-fertilisation increased not only the total S-content, as well as S-containing compounds such as cysteine, GSH, and GSL in oilseed rape but moreover also caused changes in the composition, oviposition behaviour, performance and development of adults and larvae of different species of oilseed rape visiting insects. These compounds act as feeding deterrents for polyphagous herbivores and as feeding stimulants for crucifer-specialists. Therefore S-fertilisation of oilseed rape may be a measure to control generalist herbivores and probably decrease the occurrence of *Meligethes* spp. at early flowering but for specialist insects S-fertilisation can be not used to control infestation. It was shown that S-application increased the population of specialist feeders of oilseed rape at various growth stages. A higher level of N- application seems to increase the susceptibility of oilseed rape for various pest organisms therefore a balanced fertilisation between S and N is most recommendable for highly productive oilseed rape cropping.

5 Summary

Oilseed rape is a widely grown crop with a high S-demand and therefore S-fertilisation belongs to the common fertilisation practice to achieve high yields. However such applications does not only affect the productivity of the crop but also the population dynamics of beneficial and pest insects. The S-nutritional status is affecting the crop in two ways: it has an influence on morphological features such as the size and form of flowers and inflorescences and the colour of the flowers and on the other hand the S-status is affecting the composition of the crop by altering the concentration of primary and secondary S-containing compounds such as the GSL-content. Up to now only a limited number of studies were conducted which investigated the influence of the S-nutritional status of the crop on the composition of oilseed rape visiting insects. Therefore it was the main target of this work to investigate if the S-nutritional status of oilseed rape is affecting the composition of insects and if the infestation level with several pest organisms was changed in relation to the S-nutritional status. Additionally the influence of N-nutrition was also tested because of the close relationship between N and S in plant metabolism.

In this context different trapping methods were used and investigated for their suitability to monitor the population dynamic of different insects in relation to S- and N-nutrition.

The main results of the present work were:

1. S-fertilisation increased the total S-content of the plant as well as primary (cysteine and GSH) and secondary (GSL) S-containing constituents.
2. High rates of N-fertilisation increased significantly the total N-content in young leaves.
3. N-fertilisation had a positive influence on the population dynamics of most investigated insect species.
4. Experimental conditions such as the size of the plots, the grown cultivar of oilseed rape as well as the surrounding landscape and the methods to monitor the infestation of oilseed rape with several insect pests are of major relevance for the results of such experiments where insects were classified in relation to nutritional factors of the crop.
5. With the cluster analysis it was possible to classify the oilseed rape visiting insects into specialists and generalists on the basis of their relation to the S-nutritional status of the crop. In general S-application decreased the density of generalist insects while the population of specialist feeders increased with S-fertilisation most likely because of the increasing GSL-content where specialists are adapted to.

Generalist insects:

1. From the generalist insects adults of *Delia platura* significantly decreased with S-application at their peak of occurrence but a decrease with S-fertilisation was not observed at all growth stages of oilseed rape. S-fertilisation also significantly decreased the infestation of oilseed rape with adults of *Scaptomyza flava* especially at the two peak times of occurrence.
2. Adults and larvae of *Staphylinidae* family, *Tachyporus* genus and the dynamic population of *Syrphidae* as polyphagous predators were only indirectly influenced by S-nutrition by the effect of S-content in their prey.
3. S-application increased the density of spiders which belong to the polyphagous predators at early spring while at other growth stages of oilseed rape the population decreased.

Specialist insects:

1. Regarding to the infection of oilseed rape by *Meligethes* spp., S-application increased the occurrence of adults and larvae of *Meligethes* at main flowering while the population decreased at early flowering.
2. The oviposition behaviour of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* as well as the feeding damage by larvae of both species significantly increased with S-fertilisation in early spring.
3. Infection rates by adults and larvae of *Ceutorhynchus obstrictus* were found to be significantly higher with S-fertilisation during most growth stages of oilseed rape.
4. Adults and larvae of *Dasineura brassicae* positively responded to higher GSL-contents in S-fertilised plots especially when their population reached their peak of occurrence for the first and second generation.
5. S-fertilisation increased slightly but not significantly the infestation level with adults of *Delia radicum* at most growth stages of oilseed rape.
6. *Brevicoryne brassica* showed a positive response to S-fertilisation at pod development.

S-fertilisation can improve the resistance of oilseed rape against generalist pests of oilseed rape through enhancing defence compounds in the plant. On the other hand these compounds act as feeding and oviposition stimulants and they improve host plant location for crucifer-specialists which are well adapted to these compounds. Therefore S-fertilisation seem to be no good measure to control infestation of oilseed rape with insect pests especially as most of the specialist insects cause more serious damage to the crop compared to generalist insects.

Therefore application of the appropriate rate of S is recommended to obtain high yields and vital plants which are probably also more resistant against fungal diseases. Furthermore a controlled application of N seems to be most recommendable as high doses of N increased the infestation with most insect pests. Despite of the fact that many different factors are affecting the population dynamic of pest insects in this work a clear relationship between nutritional factors and the infestation level with certain pest was shown for S as well as for N.

Zusammenfassung

Raps ist eine weit verbreitete Feldfrucht mit einem hohen Bedarf an S. Folglich gehört die S-Düngung zur allgemeinen Düngepraxis, um hohe Erträge zu sichern. Die S-Düngung beeinflusst aber nicht nur den Ertrag von Raps, sondern auch die Lebensgemeinschaften von nützlichen Insekten wie auch von Schadinsekten. Hierfür sind vor allem zwei Mechanismen zu nennen, diesichbei Raps in Abhängigkeit von der S-Versorgung ändern: zum einen hat S Auswirkungen auf morphologische Parameter wie Größe und Form der Blüten und des Blütenstandes sowie auf die Blütenfarbe. Zum anderen beeinflusst die S-Versorgung die Zusammensetzung von Inhaltsstoffen durch Veränderung der Primär- und Sekundärmetabolite, wie z. B. dem GSL-Gehalt. Bis zum heutigen Zeitpunkt existieren nur wenige Studien, die den Einfluss der S-Versorgung von Raps auf die Biodiversität von pflanzenbesuchenden Insekten untersucht haben. Das Hauptziel dieser Arbeit war es, zu betrachten, ob die S-Versorgung einen Einfluss auf die Biodiversität von Insekten bei Raps ausübt und bei einer Änderung der Biodiversität den Einfluss der S-Versorgung auf ausgewählte Schadinsekten genauer zu betrachten. Aufgrund des Zusammenspiels von S und N im pflanzlichen Metabolismus wurde neben der S- auch die N-Versorgung der Pflanzen berücksichtigt. Für die Untersuchungen wurden verschiedene Fangmethoden angewandt und deren Eignung für das Monitoring von Insekten in Abhängigkeit ihres Lebenszyklus und von der S- und N-Versorgung betrachtet.

Die durchgeführten Untersuchungen führten zu folgenden Ergebnissen:

1. Die S-Düngung führte zu einem Anstieg des S-Gehaltes und der gemessenen S-haltigen Primär- (Cystein und GSH) und Sekundärmetabolite (GSL) in den Pflanzen.
2. Eine hohe N-Versorgung führte zu einem signifikant höheren N-Gehalt in jungen Rapsblättern.
3. Die N-Versorgung hatte einen positiven Einfluss auf den Lebenszyklus der meisten untersuchten Insektenarten.
4. Versuchsbedingungen wie Parzellengröße, Rapssorte, die umgebende landschaft und die angewandten Methoden zum Monitoring des Insektenbefalls an Raps mit unterschiedlichen Schadinsekten, besitzen einen großen Einfluss auf die Zusammensetzung der Insektengemeinschaft in Abhängigkeit von der Nährstoffversorgung der Pflanzen.

5. Durch die durchgeführte Clusteranalyse konnten die rapsbesuchenden Insekten in Abhängigkeit von der S-Versorgung in Spezialisten und Generalisten unterschieden werden. Im Allgemeinen führte die S-Düngung zu einer Abnahme der Generalisten, während spezialisierte Arten durch einen Anstieg des GSL-Gehaltes bei höherer S-Versorgung zunahmen.

Generalisten:

1. Von den Generalisten verringerte sich die maximale Anzahl der Adulten von *Delia platura* signifikant bei erfolgter S-Düngung. Eine Abnahme ihres Vorkommens in Abhängigkeit von der S-Düngung wurde nicht in allen Wachstumsstadien des Raps beobachtet. Des Weiteren bewirkte die S-Düngung eine Verringerung des Befalls mit Adulten von *Scaptomyza flava* besonders zu den zwei Hauptzeiten des Auftretens an Rapspflanzen.
2. Adulte und Larven der Familie *Staphylinidae*, Klasse *Tachyporus,* sowie die Anzahl von *Syrphidae* als polyphage Räuber wurden nur indirekt von der S-Versorgung beeinflusst durch die Wirkung von S auf ihre Beute.
3. S-Düngung erhöhte die Dichte der Spinnen, die zu den polyphagen Räubern im Frühjahr gehören. Während der anderen Wachstumsstadien des Raps verringerte sich hingegen die Spinnenanzahl.

Spezialisten:

1. In Bezug auf den Befall der Rapspflanzen mit *Meligethes* spp., führte die S-Düngung zu einem Anstieg des Vorkommens von Adulten und Larven von *Meligethes* spp. während der Hauptblüte. Zu Beginn der Blüte war die Population hingegen geringer.
2. Das Eiablageverhalten von *C. napi* und *C. pallidactylus,* sowie die Fraßschädigung der Pflanzen durch die Larven beider Arten, erhöhte sich erheblich nach erfolgter S-Düngung im Frühjahr.
3. Die Befallsstärke durch Adulte und Larven von *C. pallidactylus* war während der meisten Wachstumsstadien höher in Parzellen mit erfolgter S-Düngung.
4. Das Auftreten von Adulten und Larven von *Dasineura brassicae* zeigte einen positiven Zusammenhang mit der S-Versorgung und mit dem höheren GSL-Gehalt der Pflanzen, insbesondere zum Zeitpunkt der höchsten Populationsstärke für die erste und zweite Generation.

5. Die S-Düngung erhöhte sichtlich, wenn auch nicht signifikant den Befall des Raps mit Adulten von *Delia radicum* während der meisten Wachstumsstadien.
6. *Brevicoryne brassica* zeigte hingegen einen positiven Zusammenhang zwischen der Befallsstärke und der S-Versorgung zum Zeitpunkt der Schotenbildung.

Die S-Düngung kann die Resistenz von Rapspflanzen gegen Generalisten unter den Schadinsekten durch die Steigerung von Abwehrverbindungen in den Pflanzen erhöhen. Auf der anderen Seite wirken diese Verbindungen anziehend auf Spezialisten, die an diese Verbindungen gut angepaßt sind und deren Vorkommen und Eiablage dadurch gefördert werden. Folglich ist die S-Düngung nicht sehr gut für die Kontrolle der Befallsstärke von Schädlingen geeignet, da die meisten spezialisierten Arten zu einer höheren Schädigung der Rapspflanzen im Vergleich zu den Generalisten führen. Daher ist die Düngung nur dafür geeignet, einen hohen Ertrag zu erzielen und einen gesunden Bestand, der vermutlich resistenter gegen Pilzerkrankungen ist, zu gewährleisten. Des Weiteren ist eine kontrollierte Versorgung mit N anzustreben, da die Ergebnisse zeigen, dass eine hohe N-Versorgung zu einer Erhöhung aller Schädlinge führte. Trotz der Tatsache, dass viele Faktoren die Dynamik der Schadinsekten beeinflussten, ist innerhalb dieser Arbeit ein klarer Zusammenhang zwischen den Nährstoffen S und N und der Befallsstärke mit bestimmten Schädlingen zu erkennen.

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7 Glossary

Abiotic: Inanimate environmental factors such as climate, temperature, etc., that do not derive directly from the presence of other organisms.

Allelochemicals: A substance produced by one organism that is toxic or inhibitory to the growth of another

Anemotaxis: The ability of certain insects to orient themselves in relation to wind direction.

Arrestant: A chemical or physical source that causes an organism to aggregate in contact with it. The aggregation near source by kinetic responses.

Attractant: orientation to source.

Deterrent: prevention of continued feeding or oviposition or hastening of their termination.

Glucosinolates: The low molecular mass nitrogen and S containing secondary compounds.

Glutathione: is a molecule consisting of 3 amino acids that is produced in the livernts and in pla.

Gravid: This term usually is restricted to females that having the body distended with ripe eggs.

Isothiocyanates: an unstable intermediate that undergoes nonenzymatic rearrangement form sulfate and isothiocyanates

Monophagous: Insect feed upon a single kind of food.

Neoplasm: A new growth of tissues or cells, such as a tumor, serving no physiological function.

Oligophagous: Insect feed on few kinds of food.

Phytoalexins: group of compounds that occur naturally in all fruits and vegetables. They are now thought to offer degree of protection against cancer, heart disease, arthritis, hypertension and other degenerative ailments. They are by definition secondary metabolites synthesised de novo by plants in response to diverse forms of stress

Phytophagous: many taxa contain individuals which are relatively restricted in the kinds of plants they eat or on which they lay eggs, while far fewer are catholic in their choice of suitable resources. Phytophagous insects include monophagous and /or oligophagous

Polyphagous: Insect capable of consuming many types of food material.

Repellent: movement away from the source.

Stimulant: promotion of continued feeding or oviposition or promotion of biting or probing .

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8 Appendix

I Key to the Orders of Insects

1a Insect with wings ....……….....................……..2
1b Insect without wings ....…......................………...................………........……......20

2a Insects with four wings (two pairs). ……..................................……………………....3
2b Insects with only two wings (one pair).……..……………....18

3a Wings covered with scales such as butterflies and moth …......…………**Lepidoptera**
3b Wings not covered with scales, though they may be hairy...…...…....………….....…4

4a Forewings partly or entirely horny or leathery and used as covers for hind-wings, often much narrower than hind wings…………...……..……………………………..5
4b both pairs of wings entirely membranous (flexible) and used for flying.....………...…9
5a Mouthparts are tube-like, adapted for piercing and sucking as true bugs......**Hemiptera**
5b Mouthparts are adapted for biting and chewing..…………..…...........………..……...6

6a Forewings and hind-wings with veins, hind-wings stiffer and harder than forewings and serving as covers for hind-wings.
6b Fore-wings without veins, and modified into hard, horny cases for hind-wings……....7

7a Fore wings are short.
7b Fore wings as long as, or nearly as long as abdomen, the two wings may be joined. They meet along the animals back and hence are never used for flying …...**Coleoptera**

8a End of abdomen with characteristic pair of forceps like cerci (Earwigs)…**Dermaptera**
8b End of abdomen without characteristic forceps like cerci such as Beetles…………… ……………………………………….……………….**Coleoptera, Staphylinidae**

9a Wings narrow and without veins, but fringed with long hairs. Very small insects, about 5 mm in length……..….......…………………….………..…………………..**Thysanoptera**
9b Wings more fully developed, and with veins present……..………………..................10

10a Hind-wings noticeably smaller than forewings......……………….….....………......11
10b Hind-wings similar in size to or larger than fore-wings…………...…….……………15

11a Abdomen has two or three long 'tails'. Forewings with a large number of cross-veins, making a net-like pattern like Mayflies...…………..………………..... **Ephemeroptera**
11b Forewings have fewer veins, not forming a net-like pattern, usually without 'tails' ...12

12a Wings obviously hairy. Mouthparts are very small, except forpalpi such as Caddis flies……………………….. …………………………………………**Trichoptera**
12b Wings not obviously hairy, though tiny hairs can be seen under the microscope ..….13

13a Mouthparts well developed and adapted for biting and chewing.…………………...14
13b Mouthparts tube-like, adapted for piercing and sucking such as Aphids; Cicadas etc ....…………………………………………………....**Hemiptera; Homoptera**

14a Very small insects, soft-bodied, mostly less than 6 mm in length, tarsi with only two or three segments...............……………...................................……………….…………...15
14b Often much bigger, wasp-like or bee-like insects; or if very small, then hard-bodied, with abdomen narrowed at its base into a petiole, or 'waist', tarsi with four or five segments such as Bees, Wasps, Ants and Sawflies……….…………..**Hymenoptera**

15a Tarsi only have three or four segments.
15b Tarsi with five segments.......……..…....………...……....16

16a Mouthparts prolonged into a beak such as Scorpion flies..………………... **Mecoptera**
16b Mouthparts short..........................………..…….………..17

17a Most of the veins in forewings divide or fork just before they reach the wing edge, hind-wings broader than forewings at least at base such as Alderflies, Snake flies..…..……......……….............….**Megaloptera**
17b Few or no veins in the forewings fork immediately before the wing edge hind-wings similar to forewings............……………..……………………………….. **Neuroptera**

18a Hind-wings absent or reduced knob-like organs (called halters).…..……...…….........19
18b Forewings absent or reduced to knob-like organ………………………….**Strepsiptera**

19a Hind-wings reduced or modified to knob-like organs (called halters), mouth-parts with various forms such as True Flies………………..…...……… …………...…..**Diptera**
19b Hind-wings entirely absent; no halters such some of Mayflies…...……**Ephemeroptra**

20a Some segments with jointed legs, which can be used for movement……………......21
20b No jointed legs; or if these are present and visible, then they are enclosed in membrane, and cannot move...……………………..Larvae and pupae of **Endopterygota**

21a Animals found living as parasites on warm-blooded animals, or found closely associated with them in their nests or dens.
21b Animals not found living as parasites on warm-blooded animals: either free living, or parasitic on other insects, snails etc…………..…...............................….................. 22

22a Terrestrial: living on dry land, or on animals other than mammals and birds………..23
22b Aquatic: mostly nymphal forms of terrestrial insects.

23 Mouthparts not visible, abdomen with appendages on some of the abdominal segments, or with a forked 'spring' near tip.............…………………..…...........…..24
23b Mouthparts clearly visible................…....…..........……...25

24a Abdomen has six segments or fewer, usually with a forked appendage ('spring') near tip, no long bristles at tip of abdomen such as Springtails………………… **Collembolla**
24b Abdomen has nine or more segments, no spring, but several segments have simple appendages.

25a Mouthparts mostly adapted for piercing or sucking...........................……………..26
25b Mouthparts not as above, adapted for biting and or chewing..….......…...........….......30

26a Body covered with scales or dense hairs such as Wingless Moths………. **Lepidoptera**

26b Body bare or with few scattered hairs ....…….............…..……...........................27

27a Almost all of thorax that is visible above is composed of the middle segment, the mesothorax: prothorax and metathorax both small and hidden such as wingless True flies...…….………………………………………………………………….. **Diptera**

27b Mesothorax and metathorax about equally developed, prothorax also is usually visible from above………..…………..…...……....28

28a Snout (proboscis) is small, cone-shaped, body long and narrow, claws and usually absent such as Thrips…….……………………………………………... **Thysanoptera**

28b Snout (proboscis) is longer, jointed. Body more or less oval, claws present………....29

29a Proboscis is arising from front part of head. Abdomen without cornicles near tip such as wingless bugs……...……………………………………….……………...**Hemiptera**

29b Proboscis is arising from hind part of head. Abdomen is often with two cornicles at or near its tip like Aphids………….………………………… **Hemiptera; Homoptera**

30a Abdomen has false or pro-legs, which are fleshy and different from the jointed legs of the thorax like Caterpillar.

30b Abdomen without any kind of legs, only thorax has legs..........………...............…..31

31a Antennae are indistinct……………………………………….………….……… Larvae

31b Antennae are long and distinct. Adult insects.....………..32

32a Abdomen has a pair of movable forceps like cerci at tip such as Earwigs..**Dermaptera**

32b Abdomen without such forceps......………...........................…..............................33

33a Abdomen strongly constricted at base into a 'waist'. Sometimes antennae are bent into an elbow such as Ants and wingless Wasps………...………………...…**Hymenoptera**

33b Abdomen not constricted into a waist.

Morphological Keys to Coleopteran families and species (adapted from Klimaszewski and Watt, 1997.

1a Metacoxae large fused to metasternum, completely dividing 1st ventrite; sternites13 fused; prothorax with distinct notopleural suture **Adephag**…………………………...2

1b Metacoxae varying in size, usually movably articulated to metasternum, never completely dividing 1st ventrite; all sternites usually free; prothorax without notopleural sutures **Polyphaga**.……..……………………...…………………….…….4

2(a) Hind legs without swimming hairs; prosternum not prolonged behind as a median keel; terrestrial beetles not streamlined for aquatic life. ……………………..……....3

3(a) Antennae usually filiform never moniliform; head and pronotum without deep, paired, longitudinal grooves *(Carabidae)* pronotum with 2 lateral setae, one of them at hind-angles, head mostly with 2 supra-orbital punctures..................................... (***Amara***) 40

4(a) Antennae with 10 or fewer segments, the terminal segments lamellate, produced anteriorly into laterally flattened plates such as ***Scarabaeoidae***………………..……...5

4(b) Antennae rarely lamellate or flabellate, if so then 11-segmented ….…………...……. 7

5(a) Antennae not geniculate, with club segments to be folded closely together; mandibles not prominent………..……………………………..…………………………………6

6(a) Abdomen with 6 ventrites; elytra smooth, or if it was sculptured then not rough …………………………………………………………….……………… ***Scarabaeidae***

7(a) Tarsi pseudotetramerous (5-5-5 but appearing 4-4-4), with third segment usually strongly bilobed, rarely pseudotrimerous (4-4-4- but appearing 3-3-3-) ***Curculionoidea, Chrysomeloidea***……………...…………………..………..……...…8

7(b) Tarsi are not so………………………………….……………………..……………...13

8(a) Antennae and pronotum not like Chelonariidae (antennae lamellate, pronotum humped laterally)…………………………...…………..…………………...……………..…9

9(a) Head without a rostrum, or rarely slightly rostrate; antennae without a club, not geniculate; antennal scrobes absent; gular sutures distinct and separate such as Chrysomeloidea..………………...……………..……………………………………..10

9(b) Head usually produced into a rostrum; antennae with a more or less distinct club, often geniculate and retractable into scrobes on side of rostrum; gular sutures usually confluent or obsolete such as ***Curculionoidea*** …………….…………………..……......12

10(a) Antennae not inserted on tubercles, not capable of being flexed backwards against body, usually not extending to the base elytra....…………..……..…………………..11

11(a) Head somewhat rostrate; antennae and body bearing scales such as ***Bruchinae*** ………...………………...……………………………………………***Chrysomelidae***

11(b) Head not at all rostrate; antennae and body without scales such as ***Chrysomelidae***…38

12(a) Antennae usually geniculate, with 1st segment retractable into scrobes such as ***Curculionidae***….………………………………………………………….…..…...34

13(a) Antennae rarely geniculate, or if so then without compact 3- segmented club; other characters never all present in combination ……………...…………...……………...14

14(a) Metacoxae with posterior face vertical and at least slightly, usually strongly, excavate to receive retracted femur; antennae filiform, serrate, pectinate, or thickend but never with a true club; ocelli absent, procoxal cavities open behind……………….…….15

15(a) Abdomen with all ventrites usually free, or if fused (Elateroidea) then suture between 1st and 2nd ventrites as distinct as that between 2nd and 3rd; tarsi rarely with adhesive lobes on more than 1 segment body from not as above………..……………………..16

16(a) Anterior median part of mesosternum deeply and narrowly excavate, with side of activy vertical, receiving narrow, pointed posterior process of prosternum, these together usually forming part of "clickmechanism"; abdomen with basal 4 ventrites fused; body form characteristic, hind angles of pronotum almost always produced backwards, partly around elytra shoulders such as ***Elateroidea*** …...….………………..17

16(b) Anterior median part of mesosternum shallowly and broadly excavate, or not excavate at all; posterior prosternal process absent, or not shaped as above; abdominal ventrites free; body form not as above; hind angles of pronotum at most rectangular, not produced backwards……….…………..………………………………………...18

17(a) Labrum free, visible externally; antennae inserted near eyes…..……..…….***Elateridae***

18(a) Elytra truncate, leaving usually 6 sternites exposed …..……….. (in part) ***Staphylinida***

19(a) Antennae short, with 6th segment modified as a cupule and terminal 3 or 4 segments forming a strong, pubescent club, or if club weak and not pubescent, then maxillary palps much longer than antennae; head often with a Y-shaped impressed line on vertex.

19(b) Antennae no as above, longer than maxillary palps; head without a Y-shaped impressed line……………………………………………….……………………...20

20(a) Elytra truncate at apex, leaving at least 3 sclerotised abdominal tergites uncovered; antennae fliform or thickened towards apex, but without a strong, compact club……21

20(b) Head without this combination of foveae and lateral incisions…………....………...22

21(a) Abdomen with very limited dorsoventral flexibility; maxillary palps are usually long and modified; integument with characteristic deep foveae in various positions, especially on vertex of head and pronotum such as…….. ***pselaphinae (Staphylinidae)***

21(b) Abdomen flexibile dorsoventrally; maxillary palps usually moderately long and not modified; integument rarely with such foveae such as ***Staphylinidae***……….….…...41

22(a) Metacoxae with posterior face not vertical……………..……...…..………...…23

23(a) Tarsal formula not 5-5-4…………………………………………….……..…...24

24(a) Tarsal formula 5-5-5……………..………………………………………..……...25

25(a) Metasternum shorter than combined length of ventrites 1-4; legs longer; body shape not so……………………………………………………………………...……...26

26(a) Antennae with last 5 segments distinctly broader than basal segments, forming a loosely articulated club, with segment 8 smaller than 7 or 9, or rarely with a 4-segmented club with segment 8 smaller than segment 9, 10, or 11, if antennae fliform then elytra with transverse striae; protarsi broader in males than in females; body moderately to strongly conve, oval in outline……...……………..……***Leiodidae***

26(b) Antennae not so, elytra without transverse striae; protarsi usually not broader in males than in females……………….………….……………………………...……….27

27(a) Antennae not like Agyrtidae (weakly but distinctly clubbed, with segment 1-6 glabrous, segment 3 longer than scape, segments 4-11 much broader), protarsi and mesotarsi rarely expanded in males…………………………..……………………...28

28(a) Body small, less than 2.5mm long, glossy, very convex; elytra truncate apically, exposing sclerotised pygidium; ventrite 1 at least as long as next 3 ventrites together such as………………………………………….….***Scaphidiinae (Staphylinidae)***
28(b) Body usually larger, if small then not glossy and strongly convex; elytra not truncate, pygidium not sclerotised; ventrite 1 shorter than next 3 ventrites……….………..…29

29(a) Body not shaped, if abdomen with pygidium exposed and sclerotised then antennal club 3-segments and body less elongate ……………...…………………..……….30

30(a) Antennae with a broad, compact, 3-segmented club; pygidium and sometimes 1 or 2 tergites in front of it, usually sclerotised and exposed such as ***Nitidulidae***…….….…31

31(a) Labrum free; procoxae open or closed; tegmen with or without lateral lobes……..……..……………………………………………………………...…32
31(b) Labrum and frons fused; procoxae open; tegmen without lateral lobes ………………………………………………………………...…...***Cryptarchinae***

32(a) Base of pygidium and frequently base of last visible sternite with a pair of semicircular impressed lines; intermediate and hind tibiae strongly depressed, with single marginal carina on outer margin; outer edge of anterior tibiae often toothed such as ***Meligethianae***..……….……………………………………………..……………. 33

33(a) Last visible abdominal sternite with distinct, impressed, semi-circular lines; both sexes with a compact, three-segmented club…..…..………………………….***Meligethes* spp.**

34(a) body more-or-less quadrate and often at least partly clothed in hair-like scales, 2-4mm long rostrum relatively long and trunk-like ***Ceutorhynchus***………………..…….35
34(b) ...…………………………………………………………..…………….***sitona***

35(a) legs greyish to black……………………………………….……………....….36
35(b) legs at least partly reddish………………...………..…………………...……..…...37

36(a) body 2.2-3 mm long; mainly lead-grey and relative narrow bodied, with two rows of whitish hairs between longitudinal furrows on the elytra……………………………………..….**Cabbage seed weevil (*C.obstrictus*)**

36(b) body 3.2-4 mm long; greyish, with three rows of whitish hairs between the longitudinal furrows on the elytra…....……………………**Rape stem weevil (*C.napi*)**

36(c) body smaller than cabbage seed weevil; antennae with 7 segments; with one row of whitish Hairs divided the elytra……….……………………..……….… (***C. floralis***)

37(a) body 2.5-3.5 mm long; greyish-brown, with a whitish patch of hairs just behind the thorax; legs reddish…………………………...**Cabbage stem weevil (*C.pallidactylus*)**
37(b) body 2.5-3.5 mm long; mainly shiny black, with a pale yellowish mark on the shoulder of each elytron; legs partly reddish…………...**Rape winter stem weevil (*C.picitarsis*)**

38 (a) hind legs greatly enlarged and modified for jumping; antennae filiform….……… 39
38 (b) ………………………………………………...……...……….***lema melanopus***

39(a) antennae 10-segmented; body 4-5 mm long; elytra usually metallic greenish-black or bluish black but sometimes is bronzy……………….…...…………………………….
…………………………………**Cabbage stem flea beetle** (***Psylliodes chrysocephala***)

39(b) antennae 11-segmented; body 1.5-3 mm long; elytra black or metallic greenish-black (sometimes with two conspicuous yellow longitudinal)…..……….……***Phyllotreta* spp.**

40(a) body more or less egg-shaped; pronotum with two pairs of lateral setae, one of which is situated at the hind angles; head with two supra orbital Punctures; body length 6-9 mm...…………………….……………………………………………………..***Amara* spp.**

41(a) antennae inserted on anterior margin of the head, in front of the eyes………...……..42

42 (a) distance between the antennal bases greater than the distance between the outer margins of the mandibles at their bases…..…………………..…………….……....…..43

43(a) body boat-shaped; hind part of body elongate-conical, strongly tapering towards the apex and with long setae; head retracted under pronotum, up to the eyes; pronotum shiny and mostly glabrous; antennae fliform and inserted uncovered on the anterior, descending part of the head……………..…………………….………..***Tachyporinae***...44

44 (a) terminal segment of maxillary palp minute and very short; head and pronotum glabrous; body 2-5 mm long…………...……..………………….....…...***Tachyporus* spp.**

Simple characteristics to determine the **Cabbage aphid adult (*Brevicoryne brassicae*)**

a- Adult with two pairs of membranous wings (Homoptera).

b- Body delicate, less than 3 mm long; hind end of abdomen with a pair of siphunculi and with a distinct cauda; wingless or fully winged.

c- Body greyish-green, more or less coated in mealy wax; siphunculi barrel-shaped; typically inhabiting large, dense colonies; cauda broadly triangular.

Simple characteristics to determined the **Turnip sawfly adult *(Athalia resae)***

a- Adult with two pairs of membranous wings (Hymenoptera).

b- Body robust, 6-8 mm long; mainly yellow to reddish-yellow; fore wings and hind wings fully developed; females with a saw-like ovipositor.

c- After dipterous adults were selected from other orders, the species adults identified as following morphological keys.

Morphological keys to Dipteran families and species frequently inhabiting oilseed rape fields

1 a Above the antennae there is no ptilinal suture though a formal lunule may be present. Antennae often bear flagella of many similar segments or these may be combined into a compound “third segment” – Nematocera, Brachycera, Cyclorrhapha-Aschiz …..…2

1 b Above the antennae there are distinct ptilinal suture, continued downwards on each side. Antennae are always short and three-segmented, throught the third segment nearly always bears a dorsal arista, like a long bristle – Cyclorrhapha- Schizophor ...xx

2 a Anal cell of wing is wide open, almost never narrowed towards wing margin; palpi usually with several segments, often drooping, sometimes reduced; antennae usually elongate and whip-like sometimes compact, but with distinct flagellar segments – Nematocera …………………………………………………………………….3

2 b Anal cell of wing narrowed towards margin, often closed by the meeting of veins Cu1 and A1, which then continue as a single vein; sometimes (like Empididae) this union is far back towards base of wing and combined vein may be faint or absend. Palpi with 1-4 segments, terminal one is enlarged and often porrect. Flagellar segments of antennae nearly always fused into compound third segment – Brachycera, Cyclorrhapha-Aschiza ……………………………………………………..…13

3 a At most one anal vein reaches the wing-margin, mesonotum without any V-shaped suture……………………………………………………………………..………4

3 b More than one anal vein reaches the wing margin or mesonotum with V-shaped suture ………….……………………………Families seldom inhabiting oilseed rape fields

4 a Ocelli present……….…………………………………………………………. 5

4 b Ocelli absent …………………………………………………………….…... 11

5 a Tibiae spurred at tip …….……………………………………………... ………..6

5 b Tibiae not spurred at tip ……………………………………………………..……..9

6 a Discal cell absent, wing with radial fork (if present) much beyond cross vein r-m …...7

6 b Not as above…………………………... Families seldom inhabiting oilseed rape fields

7 a Antennae in profile placed well below compound eyes, near mouth margin, shaped, with short flagellar segments closely compected; legs often strongly armoured, with conspicuous spurs and three tarsal pads (empodium and two pulvilli)……. **Bibionidae**

7 b Antennae inserted near the middle of the compound eyes or above….………………. 8

8 a Eyes “bridged”, arched together over the eyes …….……..………………….**Sciaridae**

8 b Eyes not bridged ……………………… Families seldom inhabiting oilseed rape fields

9 a Wings with reduced venation, only 4 veins reach the wing margin ….………..…….10

9 b Venation of wing not reduced ………... Families seldom inhabiting oilseed rape fields

10 a Antennae filiform, small, delicate flies …....**Cecidomyiidae** (subfamily Lestremiinae)

10 b Antennae short and compact ………………………………...………….**Scatopsidae**

11 a Wings with reduced venation, only 2-6 veins reach the wing margin. First tarsal segment very short, usually less than a quarter to the length of the second, small, delicate flies …………………………......**Cecidomyiidae** (subfamily Cecidomyiidae)

11 b At least 6 veins reach the wing margin; first antennal segment rudimentary, second more or less enlarged; first tarsal segment nearly always longer than second segment……………………………………………………………………………..12

12 a Antennae always long and delicate; Tibia without apical spurs; eyes not meeting above the antennae, m1+2 never forked, wings usually narrow ……...……….**Chironomidae**

12 b not as above……………………………Families seldom inhabiting oilseed rape fields

13(a) cleft of second antennal segment, transverse suture, and thoracic squamae all poorly developed. ……………………………………..………………………Acalyptrata

13(b) Second antennal segment is always with a distinct dorsal cleft or seam for nearly its whole length. Posterior calli or thorax differentiated, sand transverse suture often entire, or almost so; thoracic squamae usually large and concealing halteres ***Calyptrata***……………………………………………………………..……..…14

14 (a) 1- mouthparts well developed, and apparently functional; dorsum of thorax with at least a few strong bristles
2- Meropleuron usually bare, or with only soft hairs. If it has bristles, then vein M_1 is not distinctly bent forwards
3- Lower squamae are more or less conspicuous that though sometimes less projecting than upper ones. Frons of males usually narrowed, and often holoptic; frontalia often with crossed bristles
4- wing with anal veins extending to wing-margin, rarely stopping short just before this, and then frontalia with a pair of crossed bristles or setulose hairs, and at same time scutellm with fine, pale hairs beneath, at apex……………………..***Anthomiida.***

14 (b) not as above……………………...Families seldom inhabiting in the oilseed rape fields

Anthomiidae

Third and fourth wing veins parallel at their apices; arista bare or pubescent (key to group)

1(a) anterior postsutural supraalar bristle (prealar) more than half as long as the following bristle……..………………..……………………………………………………….2

1(b) anterior postsutural supraalar bristle short and fine, less than half as long as the following bristle……………..……..……………………………………………….….3

Key for males of Delia

2(a) hind femur very hairy on the basal half of the anterior surface; hind femur with 3 or 4 bristle in a short row on the apical third of the anteroventral surface…... *Delia radicum*

2(b) Hind femur not very hairy; anteroventral row of bristles longer and with stronger bristles, and extending only from about the centre to the apex, the bristles all is strong. ………..…………………………………………………………..……..*Delia florilega*

3(a) hind tibia with a comb-like series of erect, bristle-like hairs along the entire length of the posteroventral surface; basal segment of the middle tarsus with bristles that are shorter than the tarsal width.…..……………………………………………..*Delia platura*

Key for females of Delia

2(a1) Middle femur with strong anteroventral bristle near the base, the ventral bristle strong; front femur with 3 to 6 small, erect bristles on the anterior surface; front tibia with 2 posterior bristles……………..……..…………………………………......*Delia radicum*

2(b) Middle femur without an anteroventral bristle or this very weak, the ventral bristles weak; front femur usually without erect bristles on the anterior surface……………………………………………………………...….*Delia florilega*

3(1) arista not noticeably hair; abdomen grey or yellowish-grey with a darker central line dorsally; hind tibia with 1 (or 2) pre-apical bristles –like hair on the posteroventral surface…….………………………………………..…………………..…..*Delia platura*

A simple key to larvae of oilseed rape adapted from (Alford *et al*., 2003)

1 (a) three pairs of true legs present on thorax……………………………………………2
1 (b) true legs absent……………………………………….…………...……………4

2 (a) abdomen without pro legs; head orientated more- or-less horizontally and partly sunken into the pro thorax; body whitish to creamish-white; head, thoracic legs, pinacula and pro thoracic and anal plates black or brownish; not feeding externally on foliage……………………..……………….……………………...……………3
2 (b) eight pairs of abdominal pro legs; head distinct and orientated more or less vertically; body greenish-grey and later velvet-black, up to 18 mm long; feeding externally on foliage……………..……………………………………**Turnip sawfly (*Athalia rosae*)**

3 (a) up to 5 mm long; each segment with two to three brownish plates; yellowish gut contents often visible; feeding in buds and flowers……..…………...***Meligethes* spp.**
3(b) up to 8 mm long; anal plate has two upwardly curved hooks; feeding within shoots, petioles in autumn, winter and spring…………………………………………………...
…………………………………**Cabbage stem flea beetle (*Psylliodes chrysocephala*)**

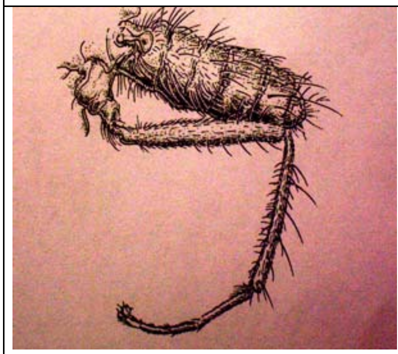
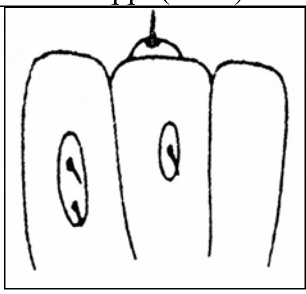
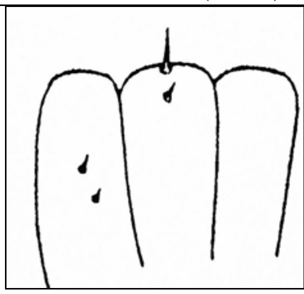
4(a) whitish to creamish-white, with a distinct, yellowish or brownish head capsule; body arched……………………...…………..……………………………...………..5
4(b) body whitish and more or less maggot-like; head indistinct…..……..………….……7

5(a) feeding, usually singly, within developing pods…..……………………...…………..…
………………………………………**Cabbage seed weevil (*Ceutorhynchus obstrictus*)**
5(b) feeding inside stems and shoots in spring and early summer; head capsule with outline of clypeus more or less triangular and the margin straight…..……..…………………6

6(a) up to 6 mm long; body whitish; spines on smooth surface of the body ………………………...……...**Cabbage stem weevil (*Ceutorhyncus pallidactylus*)**
6(b) up to 8 mm long; body creamish-white; spines on small warts elevated on surface of the body…………………………………....**Rape stem weevil (*Ceutorhyncus napi*)**

7(a) feeding gregariously within developing pods; body hyaline and translucent to whitish, up to 2 mm long..………………………..**Brassica pod midge** (***Dasineura brassicae***)

Photos of the most important insects classified in this study

 *Staphylinidae* (adult)	 *Meligethies* spp. (adult)	 *Sitona* spp. (adult)
 *C.obstrictus* (adult)	 *Ceutorhynchus floralis*(adult)	 *C.pallidactylus*(adult)
 *Tachyporus* spp. (adult)	 *Amara* spp. (adult)	 *Delia radicum* (adult)
 Hind femur of *Delia platura*	 Spines on small warts of *Ceutorhynchus napi* larva	 spines on smooth surface of *C. pallidactyllus larva*
 *Meligethies* spp. (larva)	 *C.obstrictus* larva	 *Staphylinidae* (larvae)

II Tables and figures in the appendix

Table A.1: The mineral composition of larvae of *Meligethes* spp. collected by different trapping methods in 2004.

Sampling Methods	Date	P		Mg		Ca		S		Fe		Mn		Zn		Cu		B	
		---[g kg^{-1}]---								---[mg kg^{-1}]---									
		S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}
Sweep net	04.05	20.6	27.4	11.6	16.0	37.8	50.2	19.8	26.5	427	890	79.0	78.4	384	590	191	300	744	964
	11.05	7.00	6.30	1.40	1.20	3.70	3.10	5.50	5.10	145	120	60.7	66.6	165	162	29.4	29.1	68.3	54.3
	23.05	4.60	4.80	1.20	1.20	4.10	4.30	3.50	3.80	93.0	99.3	57.6	60.1	152	456	22.4	475	39.1	37.0
Beating tray	09.05	6.20	5.80	2.20	2.10	11.0	10.6	5.00	5.10	237	254	71.9	78.5	150	160	52.6	51.6	58.6	62.4
	20.05	2.30	2.40	0.50	0.50	3.60	3.60	3.20	3.40	112	110	37.0	38.9	129	140	15.4	16.8	25.3	27.8
Suction trap	03.05	21.1	23.8	15.1	10.6	60.7	40.7	25.9	22.5	714	539	82.8	79.3	670	444	326	235	844	785
	09.05	11.3	12.6	3.90	4.70	13.5	15.0	9.90	11.2	299	447	79.9	95.2	292	267	77.7	78.5	182	244
	20.05	7.30	6.60	2.10	1.70	6.60	5.60	5.10	4.70	150	126	60.8	65.4	152	157	26.4	25.5	82.9	54.1
	31.05	7.90	8.70	6.50	5.10	26.4	19.6	9.60	8.40	1004	354	77.6	102.8	260	254	164	116	277	204
Funnel traps	24.05	4.70	4.80	-	-	4.80	5.60	3.80	4.20	-	-	-	-	-	-	-	-	-	-
	31.05	7.10	5.80	-	-	13.5	15.0	8.20	7.30	-	-	-	-	-	-	-	-	-	-
	17.05	5.40	6.00	2.40	3.20	9.00	12.1	4.90	5.90	160	143	52.7	64.8	162	256	62.6	52.3	111	137

S_0: without S-fertilisation (control)

S_{150}: fertilised with 150 kg S ha^{-1}

Table A.2: Influence of S-supply on the mineral composition of larvae of *Dasineura brassicae* and *Ceutorhynchus obstrictus* caughtd by funnel traps.

Kind of species	Sampling time	P		Mg		Ca		S		Fe		Mn		Zn		Cu		B	
		------------------- kg $^{-1}$]----------------------------								--------------------------------[mg kg $^{-1}$]-----------------------------------									
		S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}
Dasineura brassicae	24.05.04	5.75	5.20	2.04	1.98	11.4	12.6	6.32	6.56	188	156	24.5	22.6	221	203	46.1	38.0	239	138
	31.05.04	6.21	4.56	2.08	1.74	10.8	8.37	6.22	4.66	142	143	30.7	19.8	276	229	34.2	30.0	130	90.5
	06.06.04	5.54	5.65	1.92	1.81	8.84	6.43	5.87	5.77	207	138	27.3	20.3	299	354	45.0	30.6	106	85.2
	14.06.04	9.05	7.85	3.15	4.17	14.3	17.7	9.73	10.5	316	740	54.6	35.2	225	223	74.6	81.1	256	402
	28.06.04	2.68	2.48	-	-	6.83	4.04	4.69	4.15	-	-	-	-	-	-	-	-	-	-
	05.07.04	3.08	2.81	-	-	4.88	3.58	4.26	4.02	-	-	-	-	-	-	-	-	-	-
	12.07.04	2.92	4.50	1.93	2.15	11.2	10.3	4.96	5.68	244	279	19.5	29.2	182	160	31.3	36.7	142	169
Ceutorhynchus obstrictus	06.06.04	3.49	3.88	1.83	1.97	2.74	2.89	2.81	3.06	122	128	88.6	100	150	172	12.2	15.5	46.1	67.1
	21.06.04	6.37	5.97	3.67	3.36	12.3	9.80	4.56	4.20	228	175	113	119	197	180	27.5	27.3	223	136

Table A.3: Influence of S-fertilisation on the mineral composition of larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactyllus*

Pest species name	P		Mg		Ca		S		Fe		Mn		Zn		Cu		B	
	-------------------------[g kg $^{-1}$]----------------------------								--------------------------------[mg kg $^{-1}$]-----------------------------------									
	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}	S_0	S_{150}
Ceutorhynchus napi	7.30	6.00	2.37	2.15	1.61	1.65	3.81	3.85	110	120	52.0	59.2	241	170	24.8	23.6	43.5	43.5
C. pallidactylus	14.6	17.2	2.67	3.08	2.20	2.59	5.58	6.88	124	142	97.1	96.8	216	228	32.8	38.3	65.7	64.7

S_0: without S-fertilisation; S_{150}: fertilised with 150 kg S ha^{-1}. All larvae were collected by stem dissection (18.05.04).

Table A.4: The number of adults of *Meligethes* spp. and relative infection rate (RIR) in relation to S- and N-application and cultivars of oilseed rape at different growth stages (%).

Trapping method	Factor		Relative changes in the occurrence of adults of *Meligethes* spp. at different growth stages (BBCH) relative to control (%) in 2004															Whole season
			61	63	64	65	66	69	71	72	75	76	78	81	83	86	89	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	S0	104	362	113	110	52		22.3		4.63	1.25						770
		S150	97	334	19	112	70		26.6		2.88	1.88						767
		RIR %	**-6**	**-8**	**+5**	**+2**	**+34 ***		**+20**		**-38**	**+50**						**-0.49**
Suction trap (adults/ 20 plants)		S0	37.1			37.9	13.5	11.0		4.38	2.13			0.25				108
		S150	37.0			28.4	13.1	10.6		4.38	0.50			0.38				97.5
		RIR %	**+1**			**-25**	**-3**	**-3**		**0**	**-76**			**+50**				**-9**
Beating tray (adults/ 20 plants)		S0			64.5		47.5	20.0										132
		S150			74.6		68.1	16.4										159
		RIR %			**+16**		**+43**	**-18**										**+21**
Emergence traps (adults/ m^2)		S0									1.00	22.5	268	33.5	5.00	1.52	0.52	332
		S150									0.52	26.5	202	62.0	4.25	0.52	0.52	296
		RIR %									**-50**	**+18**	**- 25**	**+85**	**-10**	**-67**	**0**	**-11**

Table A.4: continued

Trapping method	Factor		Relative changes in the occurrence of adults of *Meligethes* spp. at different growth stages (BBCH) relative to control (%) in 2005																Whole season
			53	62	64	66	71	72	75	76	77	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	S0	0.75	50.5		30.5	33.5		72.1	13.5		2.50	6.38	9.25	2.00		0.25	0.13	221
		S150	0.75	34.0		37.1	125		69.3	14.4		14.0	13.0	40.6	4.63		1.00	0.25	354
		RIR %	**0**	**-33**		**+22**	**+272** ***		**-4**	**+6**		**+460** ***	**+104** **	**+339** **	**+131** *		**+300** *	**+100**	**+60** *
Suction trap (adults/ 20 plants)		S0	1.13		11.3	11.5		6.63		2.50	0.75	2.63		1.88					38.3
		S150	0.50		10.9	22.0		13.6		11.3	6.38	12.9		0.50					78.0
		RIR %	**-56**		**-3**	**+91** **		**+106** **		**+350** ***	**+750** **	**+390** **		**-73**					**+104** **
Beating tray (adults/ 20 plants)		S0		49.8		21.5	28.5		31.3	4.25	0.50	0.38							136
		S150		50.6		30.0	65.3		97.5	10.1	5.13	5.88							265
		RIR %		**+3**		**+40**	**+129**		**+212** **	**+138** *	**+925** **	**+1467** ***							**+95** **
Emergence traps (adults/ m^2)		S0											97.2	12	3.52	1.00	1.25	5.0	120
		S150											107	67.2	15.0	1.52	0.0	0.0	190
		RIR %											**+10**	**+458**	**+329**	**+50**	**-100**	**-100**	**+59**

Table A.4: continued

Trapping method	Factor		53	62	64	66	71	72	75	76	77	78	81	83	86	89	97	99	Whole season
			Relative changes in the occurrence of adults of *Meligethes* spp. at different growth stages (BBCH) relative to control (%) in 2005																
Sweep net (adults/ 40 sweeps)	Nitrogen [b]	N100	0.63	44.8		34.1	60.1		65.3	10.5		5.50	11.0	19.0	3.63		0.63	0.13	225
		N200	0.88	39.8		33.5	97.9		76.1	17.4		11.0	8.38	30.9	3.00		0.63	0.25	320
		RIR %	**+40**	**-11**		**-2**	**+63**		**+17**	**+65***		**+100**	**-24**	**+63**	**-17**		**0**	**+100**	**+25**
Suction trap (adults/ 20 plants)		N100	1.25		13.4	16.1		9.00		7.63	3.50	10.0		2.13					63.0
		N200	0.38		8.75	17.4		11.3		6.13	3.63	5.50		0.25					53.3
		RIR %	**-70**		**-35**	**+8**		**+25**		**-20**	**+4**	**-45**		**-88**					**-15**
Beating tray (adults/ 20 plants)		N100		52.0		18.3	44.5		44.5	5.00	1.75	3.38							169
		N200		48.0		33.3	49.3		84.3	9.38	3.88	2.88							231
		RIR %		**-8**		**+82***	**+11**		**+89**	**+88**	**+121**	**-15**							**+36**
Emergence traps (adults/ m^2)		N100											114	8.52	2.52	0.52	1.52	5.00	132
		N200											90.0	70.4	16.8	2.00	0	0	179
		RIR %											**-21**	**+729**	**+725**	**+300**	**-100**	**-100**	**+36**

Table A.4: continued

Trapping method	Factor		Relative changes in the occurrence of adults of *Meligethes* spp. at different growth stages (BBCH) relative to control (%) in 2004																Whole season
			61	63	64	65	66	69	71	72	75	76	77	78	81	86	89	97	
Sweep net (adults/ 40 sweeps)	Cultivar [c]	Lipton	120	359	105	104	57.5		23.6		4.75	1.50							779
		Bristol	81.0	337	127	117	63.8		25.3		2.75	1.63							758
		RIR %	**-32***	**-6**	**+21**	**+13**	**+11**		**+7**		**-42**	**+8**							**-3**
Suction trap (adults/ 20 plants)		Lipton			34.0	33.0	12.3	10.0		3.75	1.88	1.63	0.88		0.25				97.6
		Bristol			40.8	33.3	14.4	11.6		5.00	0.75	0.13	1.13		0.38				107.4
		RIR %			**+20**	**+1**	**+17**	**+16**		**+33**	**-60**	**-92**	**+29**		**+50**				**+10**
Beating tray (adults/ 20 plants)		Lipton			64.1		58.8	18.1											141
		Bristol			75.0		56.9	18.3											150
		RIR %			**+17**		**-3**	**+1**											**+6**
Emergence traps (adults/ m^2)		Lipton										0.52	31.0	257	43.6	5.52	0.52	0.52	338
		Bristol										1.00	18.0	212	52.0	4.00	1.52	0.52	289
		RIR %										**+100**	**-42**	**-18**	**+20**	**-27**	**+200**	**0**	**-15**

Relative changes in the occurrence of *Meligethes* spp. adults (%) for S-fertilised plant (S150) compared with control (S0), high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. [*]: Significant at 0.05 level; [**]: Significant at 0.01 level; [***]: Significant at 0.001 level by U-test.

Table A.5: The number of larvae of *Meligethes* spp. and relative infection rate (RIR) in relation to S- and N-application and cultivars of oilseed rape at different growth stages (%).

Trapping method	Factor		Relative changes in the occurrence of larvae of *Meligethes* spp. at different growth stages (BBCH) relative to control in 2004 (%)														Whole season
			61	62	63	64	65	66	67	69	71	72	75	76	78	81	
Pant dissection (No. of infected flowers/plant)	Sulphur [a]	S0	24.1		18.3												
		S150	23.5		16.4												
		RIR %	**-2**		**-10**												
Sweep net (larvae/ 40 sweeps)		S0				0.88	10.5	115			367		1.13	0.25			495
		S150				0.63	8.00	156			347		4.13	0.38			516
		RIR %				**-29**	**-24**	**+36**			**-5**		**+267**	**+50**			**+4**
Suction trap (larvae/ 20 plants)		S0				1.25	15.6	44.4		62.1		6.25	0.13				130
		S150				0.38	17.6	33.5		82.0		12.6	0.25				146
		RIR %				**-70**	**+13**	**-25**		**+32**		**+102**	**+100**				**+13**
Beating tray (larvae/ 20 plants)		S0				1.00		44.4		142							188
		S150				1.88		42.0		138							182
		RIR %				**+88**		**-5**		**-3**							**-3**
Funnel traps (larvae/ m^2)		S0						18	144		402	148					730
		S150						36	133		360	120					671
		RIR %						**+100**	**-7**		**-10**	**-18**					**-8**

Table A.5: continued

Trapping method	Factor		62	63	64	65	66	67	69	71	72	75	76	78	81	Whole season
			Relative changes in the occurrence of larvae of *Meligethes* spp. at different growth stages (BBCH) relative to control in 2005 (%)													
Pant dissection (No. of infected flowers/plant)	Sulphur [a]	S0	6.45	17.8												
		S150	5.0	11.8												
		RIR %	**-22 ***	**-34 ***												
Sweep net (larvae/ 40 sweeps)		S0			4.88		40.1			251		23.6	14.9	2.75	1.38	338
		S150			1.75		11.4			115		143	102	11.9	7.63	393
		RIR %			**-64***		**-72*****			**-54*****		**+505****	**+586****	**+332***	**+455***	**+16**
Suction trap (larvae/ 20 plants)		S0					12.4				7.88		6.13			26.4
		S150					33.8				14.1		33.8			81.6
		RIR %					**+173****				**+79**		**+451*****			**+209*****
Funnel traps (larvae/ m^2)		S0								145	1212	81.0	19.6	10.6		1476
		S150								39.0	703	397	192	58.6		1392
		RIR %								**-73****	**-42***	**+391*****	**+885***	**+457****		**-6**

Table A.5: continued

Trapping method	Factor		Relative changes in the occurrence of larvae of *Meligethes* spp. at different growth stages (BBCH) relative to control in 2005 (%)													Whole season
			62	63	64	65	66	67	69	71	72	75	76	78	81	
Pant dissection (No. of infected flowers/plant)	Nitrogen [b]	N100	11.0	19.6												
		N200	13.1	23.1												
		RIR %	**+9**	**+19**												
Sweep net (larvae/ 40 sweeps)		N100			4.38		30.5			194		42.8	25.8	3.25	3.50	304
		N200			2.25		21.0			172		124	91.1	11.4	5.50	427
		RIR %			**-49**		**-31**			**-11**		**+189**	**+254***	**+250**	**+57**	**+41***
Suction trap (larvae/ 20 plants)		N100					24.8				14.3		22.0			61.1
		N200					21.3				7.75		17.9			46.9
		RIR %					**-15**				**-46**		**-19**			**-23**
Funnel traps (larvae/ m^2)		N100								78.0	1081	166	48.0	28.6		1404
		N200								107	838	313	163	40.6		1464
		RIR %								**+37**	**-23**	**+90**	**+241**	**+42**		**+4**

Table A.5: continued

Trapping method	Factor		Relative changes in the occurrence of larvae of *Meligethes* spp. at different growth stages (BBCH) relative to control in 2004 (%)														Whole season
			61	62	63	64	65	66	67	69	71	72	75	76	78	81	
Pant dissection (No. of infected flowers/plant)	Cultivar [c]	Lipton	24.9		64.0												
		Bristol	22.8		34.7												
		RIR %	**-8**		**-46****												
Sweep net (larvae/ 40 sweeps)		Lipton				0.88	10.4	102			239			0.25			355
		Bristol				0.63	8.13	168			476			0.38			656
		RIR %				**-29**	**-22**	**+65***			**+99****			**+50**			**+85****
Suction trap (larvae/ 20 plants)		Lipton				1.13	16.9	44.5		61.5		4.88	0.13				129
		Bristol				0.50	16.4	33.4		82.6		14.0	0.25				147
		RIR %				**-56**	**-3**	**-25**		**+34**		**+186**	**+100**	**-19**			**+14**
Beating tray (larvae/ 20 plants)		Lipton				2.00		36.8		124							162
		Bristol				0.88		49.6		157							207
		RIR %				**-56**		**+35**		**+27**							**+28**
Funnel traps (larvae/ m^2)		Lipton						24.0	145		330	6.00	4.56				598
		Bristol						30.0	132		432	25.6	3.00				803
		RIR %						**+25**	**-9**		**+31**	**+325**	**-33**				**+34**

Relative changes in the occurrence of *Meligethes* spp. larvae (%) for: a: S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.6: Effect of S-fertilisation on the number of adults of *Meligethes* spp. caught by a suction trap during different plant growth stages of oilseed rape in 2004.

BBCH-scale	Sampling date	Cultivars	No. of adults per plant in relation to S-fertlisation (kg ha^{-1})	
			0	150
64	27.04.04	Lipton	1.61 a	1.82 a
		Bristol	2.12 a	2.13 a
65	03.05.04	Lipton	1.93 a	1.44 a
		Bristol	1.91 a	1.43 a
66	09.05.04	Lipton	0.72 a	0.62 a
		Bristol	0.73 a	0.74 a
69	20.05.04	Lipton	0.44 a	0.59 a
		Bristol	0.75 a	0.53 a
72	31.05.04	Lipton	0.23 a	0.23 a
		Bristol	0.32 a	0.31 a

No significant differences between S-application were found by using U-test at 0.05 level. n = 4.

Table A.7: Influence of S-fertilisation on the number of adults of *Meligethes* spp. caught by emergence traps during different growth stages of oilseed rape in 2003/2004.

BBCH-scale	Sampling date	Cultivars	No. of adult per 0.25 m^2 in relation to S-fertilisation (kg ha^{-1})	
			0	150
76	20.06.04	Lipton	6.32 a	9.32 a
		Bristol	5.00 a	4.00 a
78	27.06.04	Lipton	71.8 a	56.8 a
		Bristol	62.0 a	44.0 a
81	04.07.04	Lipton	8.82 a	13.0 a
		Bristol	8.00 a	18.0 a
83	12.07.04	Lipton	1.00 a	1.83 a
		Bristol	1.52 a	0.52 a

No significant differences between S-application were found by using U-test at 0.05 level. n = 4.

Table A.8: Average number of adults of *Meligethes* spp. caught with different methods in relation to S- and N-fertilisation during the season 2004/2005.

Methods	N-fertilisation (kg ha^{-1})	Number of adults at different S-supply	
		Without S	With S
Sweep net	100	237 a A	273 a A
Adult/ 40 sweeps	200	206 a A	434 b A
Suction trap	100	48.2 a A	78.5 b A
Adult/ 20 plants	200	28.5 a B	78.3 b A
Beating tray	100	132 a A	207 a A
Adult/ 20 plants	200	139 a A	323 b B
Emergence traps	100	118 a A	153 a A
Adult/ m^2	200	129.2 a A	229 a A

Mean values followed by different letters indicate significant differences by U-test at 0.05 level. n =4.

Uppercase letters are related to N-application while lowercase letters are related to S-application

Table A.9: Influence of S-fertilisation on the buds infected with larvae of *Meligethes* spp. at 21.04 2004 and 26.04 2004.

Parameters	BBCH-scale	Cultivars	S-fertilisation (kg ha^{-1})	
			0	150
% of infected buds in the main raceme	61	Lipton	23.6 a	24.6 a
		Bristol	26.1 a	21.3 a
% of infected buds in the second raceme	61	Lipton	9.41 a	9.94 a
		Bristol	13.6 a	8.64 a
No. of open flowers in the main raceme	61	Lipton	3.51 a	7.21 a
		Bristol	6.82 a	4.44 a
No. of second raceme per plant	61	Lipton	15.7 a	9.11 a
		Bristol	11.1 a	10.8 a
Total number of buds in the second raceme	61	Lipton	33.2 a	39.3 a
		Bristol	33.6 a	34.8 a
% of infected buds in the main raceme	63	Lipton	29.9 a	29.8 a
		Bristol	36.8 a	31.4 a
% of infected buds in the second raceme	63	Lipton	20.6 a	16.1 a
		Bristol	19.2 a	13.7 a

No significant differences between S-application were found by using U-test at 0.05 level. n = 40.

Table A.10: Average number of larvae of *Meligethes* spp. collected with a suction trap in relation to S-fertilisation over the whole-season 2003/2004.

BBCH	**Sampling time**	**Cultivars**	**No. of larvae per plant in relation to S-fertilisation (kg ha^{-1})**	
			0	150
64	27.04.04	Lipton	0.13 a	0.00 a
		Bristol	0.00 a	0.00 a
65	03.05.04	Lipton	0.91 a	0.82 a
		Bristol	0.71 a	1.12 a
66	09.05.04	Lipton	2.51 a	2.13 a
		Bristol	2.31 a	1.42 a
69	20.05.04	Lipton	2.62 a	3.71 a
		Bristol	3.73 a	4.62 a
72	31.05.04	Lipton	0.34 a	0.34 a
		Bristol	0.43 a	1.13 a

No significant differences between S-application were found by using U-test at 0.05 level. n = 40.

Table A.11: Numbers of *Meligethes* spp. larvae caught with a sweep net in relation to S- and N-fertilisation during different plant growth stages in 2004/2005.

BBCH	Sampling time	S-fertilisation (kg ha^{-1})	No. of larvae per 40 sweeps in relation to N-fertilisation (kg ha^{-1})	
			100	200
62	02.05.05	0	0.81 a A	0.30 a A
		150	0.25 a A	0.00 a A
64	13.05.05	0	6.53 a A	3.30 a A
		150	2.32 a A	1.30 a A
66	19.05.05	0	49.3 a A	31.0 a A
		150	11.8 b A	11.0 b A
71	26.05.05	0	241 a A	260 a A
		150	146 a A	84.3 b A
75	09.06.05	0	9.00 a A	38.3 a B
		150	76.5 b A	209 b B
76	15.06.05	0	8.80 a A	21.0 a A
		150	42.8 a A	161 b B
78	22.06.05	0	2.50 a A	3.00 a A
		150	4.00 a A	19.8 a A
81	29.06.05	0	2.50 a A	0.30 a A
		150	4.50 a A	10.8 a A
82-83	05.07.05	0	0.50 a A	0.30 a A
		150	0.30 a A	0.25 a A

Mean values followed by different letters indicate significant by U-test at 0.05 levels. n =40.

Uppercase letters are related to N-application while lowercase letters are related to S-application.

Table A.12: Average number of larvae of *Meligethes* spp. collected with funnel traps in relation to S- and N- fertilisation during different plant growth stages in 2005.

BBCH-scale	Sampling date	S-fertilisation (kg ha^{-1})	No. of larvae per trap in relation to N-fertilisation (kg ha^{-1})	
			100	200
71	25.05.05	0	10.8 a A	13.5 a A
		150	2.25 a A	4.25 a A
72	31.05.05	0	108 a A	95.0 a A
		150	72.8 a A	44.5 b B
75	07.06.05	0	4.53 a A	9.00 a A
		150	23.0 b A	43.3 b A
76	14.06.05	0	1.00 a A	2.30 a A
		150	7.00 b A	25.0 b B
77-78	21.06.05	0	1.00 a A	0.80 a A
		150	3.80 b A	6.00 a A
81	28.06.05	0	0.30 a A	0.00 a A
		150	0.00 a A	0.81 b B

Mean values followed by different letters indicate significant by U-test at 0.05 levels. n =40.
Uppercase letters are related to N-application while lowercase letters are related to S-application.

Table A.13: Average number of *Meligethes* larvae collected with different methods in relation to S- and N- fertilisation during the season 2004/2005.

Methods	S-fertilisation (Kg ha^{-1})	Number of larvae in relation to N-application	
		100 kg ha^{-1}	200 kg ha^{-1}
Funnel traps	0	125 a A	121 a A
Larvae/ trap	150	109 a A	124 a A
Sweep net	0	322 a A	358 a A
Larvae/ 40 sweeps	150	289 a A	498 b B
Suction trap	0	32.3 a A	24.0 a B
Larvae/ 20 plants	150	93.3 b A	75.5 b A

Mean values followed by different letters indicate significant differences by U-test at 0.05 levels. n =4.
Uppercase letters are related to N-application while lowercase letters are related to S-application.

Table A.14: Average numbers of *Meligethes* spp. larvae caught by different methods in relation to S-fertilisation in 2004.

Methods	Cultivars	Average numbers of *Meligethes* spp. larvae in relation to S-fertilisation (kg ha^{-1})	
		0	150
Suction trap Larvae per plant	Lipton	6.27 a	6.70 a
	Bristol	6.75 a	8.00 a
Beating tray Larvae per plant	Lipton	8.10 a	8.17 a
	Bristol	10.8 a	10.0 a
Funnel traps Larvae per m^2	Lipton	1500 a	1620 a
	Bristol	1596 a	1920 a
Sweep net Larvae per 40 sweeps	Lipton	292 a	418 a
	Bristol	698 a	615 a

No significant differences between S-application were found by using U-test at 0.05 level. n = 4.

Table A.15: The number of adults and the relative infection rate (RIR) of *Ceutorhynchus pallidactylus* collected by different methods in relation to N-application and cultivar of oilseed rape at different growth stages (%)

Trapping method	Factor		Relative changes in the occurrence of adults of *Ceutorhynchus pallidactylus* at different growth stages (BBCH) relative to control (%)																	Whole
			15	19	26	61	63	64	65	66	69	75	76	78	81	83	86	89	97	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0				4.0	1.25	0.75	0.38		0.13		0.13	6.5		1.75				14.9
		S150				5.5	2.0	0.63	0.88		0		0.25	6.75		2.00				18.4
		RIR %				**+38**	**+60**	**-17**	**+133**		**-100**		**+100**	**+4**		**+14**				**+24**
Suction trap (adults/ 20 plants)		S0						16.9	10.5	5.38	4.13	0.50	0.25		0.25		0.13			38.8
		S150						14.3	10.6	5.88	3.50	0.13	0.13		0.0		0.25			35.6
		RIR %						**-16**	**+1**	**+9**	**-15**	**-75**	**-50**		**-100**		**+100**			**-8**
Emergence traps (adults/ m²)		S0											1.00	44.4	170	36.0	15.5	13.5	1.52	282
		S150											0.0	40.4	103	80.0	18.0	11.5	2.52	256
		RIR %											**-100**	**-9**	**-39**	**+122**	**+16**	**-15**	**+67**	**-9**
Water trap (adults/ trap)	Sulphur [a] (2005)	S0	92.5	1.00	50.8															
		S150	11.8	5.30	18.8															
		RIR %	**-87**	**425**	**-63**															
Sweep net (adults/ 40 sweeps)		S0												0.63	1.38		0.13			2.13
		S150												0.13	0.38		0.00			1.38
		RIR %												**-80**	**-73**		**-100**			**-35**
Emergence traps (adults/ m²)		S0													27.0	47.2	4.52		0.52	79.2
		S150													2.52	13.0	3.52		0	23.0
		RIR %													**-91**	**-72**	**-22**		**-100**	**-71**

Table A.15: continued

Trapping method	Factor		Relative changes in the occurrence of adults of *Ceutorhynchus pallidactylus* at different growth stages (BBCH) relative to control (%)																	Whole season
			15	19	26	61	63	64	65	66	69	75	76	78	81	83	86	89	97	
Sweep net (adults/ 40 sweeps)	Cultivar ^{c} (2004)	Lipton				4.75	1.75	0.25	0.38	0.38			0.125		5.13	1.5				14.3
		Bristol				4.75	1.5	1.13	0.88	0.0			0.25		8.13	2.25				19
		RIR %				**0**	**-14**	**+350**	**+133**	**-100**			**+100**		**+59**	**+50**				**+33**
Suction trap (adults/ 20 plants)		Lipton								5.5	2.88		0.25							38
		Bristol								5.75	4.75		0.13							36.4
		RIR %								**+5**	**+65**		**-50**							**-4**
Emergence traps (adults/ m^2)		Lipton											0.52	53.2		186	65.6	16.5	18.5	342
		Bristol											0.52	32.0		87.2	50.4	17.0	6.52	196
		RIR %											**0**	**-40**		**-53**	**-23**	**+3**	**-65**	**-43**
Water trap (adults/ trap)	Nitrogen ^{b} (2005)	N100	22.4	0.75	12.6															
		N200	29.8	2.42	22.1															
		RIR %	**+33**	**+217**	**+75**															
Sweep net (adults/ 40 sweep)		N100												0.38	1.13	0.13	0.13		0.25	2.00
		N200												0.38	0.63	0.13	0		0.38	1.5
		RIR %												**0**	**-44**	**0**	**-100**		**+50**	**-25**
Emergence traps (adults/ m^2)		N100													19.5	27.0	5.52	1.52	0.52	54.0
		N200													10.0	33.0	2.52	0.0	1.0	48.0
		RIR %													**-49**	**+22**	**-55**	**-100**	**+100**	**-11**

Relative changes in the occurrence of *Meligethes* spp. larvae (%) for: a: S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.16: Relative infection rate (%) with larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* in relation to S-application and cultivar of oilseed rape.

Parameters	BBCH-scale	Factor	Main raceme	Second raceme	Total plant
Egg (Eggs/plant)	61 (2004)	S0	1.83	9.55	11.4
		S150	1.95	8.83	10.8
		RIR %	**+7**	**-8**	**-5**
Larva (Larvae/plant)		S0	0.28	0.98	1.25
		S150	0.68	0.75	1.43
		RIR %	**+145 ***	**-23**	**+14**
Oviposition holes (Holes/plant)		S0	6.03	16.8	22.8
		S150	6.83	16.8	23.6
		RIR %	**+13**	**0**	**+4**
Egg (Eggs/plant)	63 (2004)	S0	6.13	22.1	28.2
		S150	7.45	31.0	38.4
		RIR %	**+22**	**+40 ****	**+36 ****
Larva (Larvae/plant)		S0	5.35	0.3	5.65
		S150	8.68	1.38	10.1
		RIR %	**+62 ***	**+358 ***	**+78 ****
Oviposition holes (Holes/plant)		S0	11.0	6.88	17.8
		S150	10.0	4.8	14.8
		RIR %	**-9**	**-30**	**-17**
Larva (Larvae/plant)	67 (2004)	S0	24.9	32.8	57.8
		S150	23.9	29.7	53.6
		RIR %	**-4**	**-10**	**-7**
Larva (Larvae/plant)	76 (2005)	S0	0.78	0.08	0.75
		S150	0.05	0.02	0.07
		RIR %	**-93 *****	**-74 ****	**-91 *****

Table A.16: continued.

Parame-ters	BBCH-scale	Factor	Main raceme	Second raceme	Total plant
Egg (Eggs/plant)	61 (2004)	Lipton	2.00	9.48	11.5
		Bristol	1.78	8.90	10.7
		RIR %	**-11**	**-6**	**-7**
Larva (Larvae/plant)		Lipton	1.05	0.68	1.73
		Bristol	0.68	0.28	0.95
		RIR %	**-36**	**-59 ***	**-45 ***
Oviposition holes (Holes/plant)		Lipton	13.8	7.68	21.5
		Bristol	19.7	5.18	24.9
		RIR %	**+43 ***	**-33**	**+16**
Egg (Eggs/plant)	63 (2004)	Lipton	8.55	21.2	29.8
		Bristol	5.03	31.9	36.9
		RIR %	**-41 ****	**+50 ****	**+24 ***
Larva (Larvae/plant)		Lipton	5.73	1.38	7.10
		Bristol	8.30	0.30	8.60
		RIR %	**+45**	**-78**	**+21**
Oviposition holes (Holes/plant)		Lipton	9.60	6.73	16.3
		Bristol	11.4	4.95	16.3
		RIR %	**+18**	**-26**	**0**
Larva (Larvae/plant)	67 (2004)	Lipton	27.0	28.7	55.7
		Bristol	21.9	33.8	55.7
		RIR %	**-19 ***	**+18**	**0**

Relative changes in the occurrence of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* larvae (%) for: a: S-fertilised plant compared with control (without S), b- Bristol cultivars compared with Lipton.*: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by T-test.

Table A.17: Average number of adults of *Ceutorhynchus pallidactylus* collected with a suction trap and emergence traps in relation to S-fertilisation over the season 2003/2004.

Methods	**Cultivars**	**Number of adults at in relation to S-supply**	
		Without S	**With S**
Suction trap (Adults per 20 plants)	Lipton	43.3 a	32.8 a
	Bristol	34.3 a	38.5 a
Emergence traps (Adults per m^2)	Lipton	404 a	282 a
	Bristol	160 a	231 a

Table A.18: Average number of adults of *Ceutorhynchus pallidactylus* caught by emergence traps in relation to S- and N-fertilisation during different growth stages in 2005.

BBCH-scale	**Sampling date**	**Mean number of adults of *Ceutorhynchus pallidactylus* per trap**		
		S-fertilisation ($kg\ ha^{-1}$)	**N-fertilisation ($kg\ ha^{-1}$)**	
			100	**200**
81	28.06.05	0	8.75 ± 6.95 a A	4.75 ± 3.20 a A
		150	1.00 ± 0.82 b A	0.25 ± 0.50 a A
82-83	05.07.05	0	8.00 ± 8.29 a A	15.5 ± 12.9 a A
		150	5.50 ± 3.00 a A	1.00 ± 1.15 a B
85-86	12.07.05	0	1.75 ± 1.71 a A	0.50 ± 0.58 a A
		150	1.00 ± 1.41 a A	0.75 ± 0.96 a A
Whole-season		0	18.5 ± 13.3 a A	21.0 ± 15.7 a A
		150	8.50 ± 4.43 a A	3.00 ± 2.94 a A

Mean values followed by different letters indicate significant differences by U-test at 0.05 level. n = 4. Uppercase letters are related to nitrogen application while lowercase letters are related to sulphur application

Table A.19: Influence of S- and N-application on the infection of stems of oilseed rape with larvae of *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* at pod development in 2005.

Parameters	S-fertilisation (kg ha^{-1})	N-fertilisation (kg ha^{-1})	
		100	**200**
Infection (%) of main raceme	0	5.50 a A	6.03 a A
	150	2.28 b A	0.20 b B
Length of feeding tubes (cm) in the main raceme	0	6.33 a A	5.35 a A
	150	2.78 b A	0.28 b B
Number of feeding tunnel/plant in the main raceme	0	0.75 a A	0.83 a A
	150	0.20 a A	0.13 a A
Number of emergence holes/plant in the main raceme	0	0.20 a A	0.88 a B
	150	0.00 b A	0.03 b B
No. of larvae/plant in the main raceme	0	0.63 a A	0.73 a A
	150	0.10 b A	0.00 b B
Length of feeding tubes (cm) in the second raceme	0	0.18 a A	0.61 a A
	150	0.61 b A	0.14 a B
Infection (%) of second raceme	0	0.28 a A	0.98 a A
	150	0.73 b A	0.25 b A
No. of feeding tubes/plant in the second raceme	0	0.02 a A	0.01 a A
	150	0.43 b A	0.04 a A
No. of emergence holes/plant in the second raceme	0	0.00 a A	0.04 a B
	150	0.00 a A	0.00 a A
No. of larvae/plant in the second raceme	0	0.07 a A	0.09 a A
	150	0.08 b A	0.00 a A

Mean values followed by different letters indicate significant differences by U-test at 0.05 level. n = 40. Uppercase letters are related to nitrogen application while lowercase letters are related to sulphur application.

Table A.20: Average number of adults of *Ceutorhynchus obstrictus* collected by sweep net in relation to S-fertilisation during different plant growth stages in 2004.

BBCH-scale	Sampling date	Cultivars	No. of adults per 40 sweeps S-fertilisation (kg ha^{-1})	
			0	150
61	21.04.04	Lipton	9.81 a	18.8 a
		Bristol	7.54 a	13.8 a
63	25.04.04	Lipton	43.8 a	55.2 a
		Bristol	33.8 a	51.8 a
64	29.04.04	Lipton	49.5 a	39.5 a
		Bristol	36.3 a	35.5 a
65	04.05.04	Lipton	35.8 a	31.2 a
		Bristol	28.4 a	37.1 a
66	11.05.04	Lipton	26.3 a	39.3 b
		Bristol	35.9 a	34.5 a
71	23.05.04	Lipton	17.5 a	16.2 a
		Bristol	29.5 a	26.5 a
75	14.06.4	Lipton	2.82 a	2.32 a
		Bristol	2.00 a	4.31 a

Mean values followed by different letters indicate significant differences to sulphur application by U-test at 0.05 level. n = 4.

Table A.21: Average number of adults of *Ceutorhynchus obstrictus* caught with beating tray in relation to S-fertilisation during different plant growth stages in 2004.

BBCH-scale	Sampling date	Cultivars	No. of adults per 20 plants in relation to S-fertilisation (kg ha^{-1})	
			0	150
64	29.04.04	Lipton	16.3 a	12.5 a
		Bristol	10.3 a	10.5 a
67	09.05.04	Lipton	8.00 a	6.33 a
		Bristol	7.52 a	5.34 a
71	20.05.04	Lipton	12.4 a	12.8 a
		Bristol	11.8 a	11.5 a

No significant differences between treatment was found by U-test at 0.05 level. n = 4.

Table A.22: Average of number of adults of *Ceutorhynchus obstrictus* collected by emergence traps in relation to S-fertilisation during different plant growth stages (season 2003/2004).

BBCH-scale	**Sampling date**	**Cultivars**	**No. of adult per m^2 in relation to S-fertilisation (kg ha^{-1})**	
			0	150
74	11.06.4	Lipton	2.08 a	3.28 a
		Bristol	7.32 a	4.00 a
83	12.07.04	Lipton	5.28 a	2.16 a
		Bristol	3.32 a	5.28 a
86	19.07.04	Lipton	61.2 a	103 a
		Bristol	54.0 a	100 a
89	26.07.04	Lipton	55.2 a	93.2 b
		Bristol	59.0 a	133 a
97	02.08.04	Lipton	3.28 a	5.28 a
		Bristol	4.00 a	3.24 a

Mean values followed by different letters indicate significant differences to sulphur application by U-test at 0.05 level. n = 4.

Table A.23: The number of adults and the relative infection rate (RIR) of *Ceutorhynchus obstrictus* collected by different methods in relation to S- and N-application and cultivar of oilseed rape at different growth stages (%).

Trapping method	Factors		Relative changes in the occurrence of adults of *Ceutorhynchus obstrictus* at different growth stages (BBCH) relative to control in 2004 (%)															Whole season
			61	63	64	65	66	71	75	76	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	S0	8.63	38.8	42.9	31.9	31.0	23.5	2.38	0.25	0.13							179
		S150	16.3	53.4	37.5	34.0	36.9	21.3	3.25	0.13	0.13							203
		RIR %	**+88 ***	**+38**	**-13**	**+7**	**+19**	**-10**	**+37**	**-50**	**0**							**+13**
Suction trap (adults/ 20 plants)		S0					0.13				0.38	2.00						2.50
		S150					0.50				0.38	2.25						3.88
		RIR %					**+300**				**0**	**+13**						**+55**
Beating tray (adults/ 20 plants)		S0			13.3		7.75	11.9										32.9
		S150			11.5		5.75	12.1										29.4
		RIR %			**-13**		**-26**	**+2**										**-11**
Emergence traps (adults/ m^2)		S0							4.52				4.00	57.6	57.2	3.52	0.52	127
		S150							3.52				3.52	102	113.2	4.00	1.00	226
		RIR %							**-22**				**-13**	**+77**	**+98**	**+14**	**+100**	**+78 ***

Table A.23: continued.

Trapping method	Factor		Relative changes in the occurrence of adults of *Ceutorhynchus obstrictus* at different growth stages (BBCH) relative to control in 2005 (%)																Whole season
			53	62	64	66	71	72	75	76	77	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	S0	0.25	16.9		13.6	28.9		13.3	7.50		1.88	0.38	1.75	4.25		5.75	3.13	97.5
		S150	0.0	18.0		15.9	49.6		24.4	4.50		2.88	1.75	1.63	2.75		9.00	14.0	144
		RIR %	**-100**	**+7**		**+17**	**+72 *****		**+84 ****	**-40**		**+53**	**+367**	**-7**	**-35**		**+57 ***	**+348 ****	**+48 ****
Suction trap (adults/ 20 plants)		S0	10.5		10.5	3.00		1.25		0.63				0.63					26.5
		S150	4.75		15.9	5.00		4.25		2.75				0.38					33.0
		RIR %	**-55 ****		**+51 ***	**+67**		**+240 ***		**+340 ***				**-40**					**+25**
Beating tray (adults/ 20 plants)		S0		14.0			8.63		4.25	2.38	0.63	0.25							37.6
		S150		16.0			2.00		12.3	1.13	1.13	0.63							40.6
		RIR %		**+14**		**-77 ****	**-76**		**+188 ****	**-53**	**+80**	**+150**							**+8**
Emergence traps (adults/ m^2)		S0											2.52	32.5	121	29.0	2.00		187
		S150											3.00	3.00	78.0	25.0	1.52		110
		RIR %											**+20**	**-91 ****	**-36**	**-14**	**-25**		**-41**

Table A.23: continued.

Trapping method	Factor		Relative changes in the occurrence of adults of *Ceutorhynchus obstrictus* at different growth stages (BBCH) relative to control in 2005 (%)																Whole season
			53	62	64	66	71	72	75	76	77	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Nitrogen [b]	N100	0.25	23.1		16.9	40.1		19.4	3.88		1.38	0.75	0.75	3.50		8.13	6.38	125
		N200	0.0	11.8		12.6	38.4		18.3	8.13		3.38	1.38	2.63	3.50		6.63	10.8	117
		RIR %	**-100**	**-49***		**-25**	**-4**		**-6**	**+110***		**+145****	**+83**	**+250***	**0**		**-18**	**+69**	**-6**
Suction trap (adults/ 20 plants)		N100	8.00		14.1	3.38		3.25		1.25				0.63					60.6
		N200	7.25		12.3	4.63		2.25		2.13				0.38					28.9
		RIR %	**-9**		**-13**	**37**		**-31**		**+70**				**-40**					**-6**
Beating tray (adults/ 20 plants)		N100		16.4		4.00		5.88	6.38	2.38	1.63	0.38							37.0
		N200		13.6		6.63		9.13	10.1	1.13	0.13	0.50							41.3
		RIR %		**-17**		**66**		**+55***	**59**	**-53**	**-92****	**+33**							**+11**
Emergence traps (adults/ m^2)		N100											4.00	22.5	97.2	17.5	1.52		142
		N200											1.52	13.0	102	36.5	2.00		155
		RIR %											**-63**	**-42**	**+5**	**+109**	**+33**		**+9**

Table A.23: continued.

Trapping method	Factor		Relative changes in the occurrence of adults of *Ceutorhynchus obstrictus* at different growth stages (BBCH) relative to control in 2004 (%)															Whole season
			61	63	64	65	66	71	75	76	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Cultivars[c]	Lipton	14.3	49.4	44.5	33.4	32.6	16.8	2.50									193
		Bristol	10.6	42.8	35.9	32.5	35.3	28.0	3.13									189
		RIR %	**-25**	**-13**	**-19**	**-3**	**+8**	**+67**	**+25**									**-2**
Suction trap (adults/ 20 plants)		Lipton			0.50		0.5				0.38	2.25		0.25				3.88
		Bristol			0.0		0.13				0.38	2.00		0.0				2.50
		RIR %			**-100**		**-75**				**0**	**-11**		**-100**				**-35**
Beating tray (adults/ 20 plants)		Lipton			14.4		7.13	12.4										33.9
		Bristol			10.4		6.38	11.6										28.4
		RIR %			**-28**		**-11**	**-6**										**-16**
Emergence traps (adults/ m^2)		Lipton							2.52				3.52	82.0	74.0	4.00	1.00	167
		Bristol							5.52				4.00	77.2	96.0	3.52	0.52	186
		RIR %							**+120**				**+14**	**-6**	**+30**	**-13**	**-50**	**+12**

Relative changes in the occurrence of *Ceutorhynchus obstrictus* larvae (%) for: S-fertilised plant compared with control (without S), high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), Bristol cultivars compared with Lipton.*: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.24: Number of larvae and relative infection rate (RIR) with *Ceutorhynchus obstrictus* in relation to S-application at different growth stages (%) in 2004 and 2005.

Trapping method	Factor		Relative changes in the occurrence of larvae of *Ceutorhynchus obstrictus* at different growth stages (BBCH) relative to control (%)								Whole season
			72	73	75	76	78	81	83	86	
Funnel traps (larvae/m^2)	Sulphur 2004 [a]	S0	18.0	127	55.6	61.6	42.0	13.6	1.56	6.00	325.2
		S150	22.6	104	31.6	58.6	67.6	21.0	4.56	0.00	309.6
		RIR %	**+25**	**-19**	**-43**	**-5**	**+61**	**+56**	**+200**	**-100**	**-5**
Plant dissection (larvae/plant)		S0		3.65							
		S150		5.70							
		RIR %		**+56**							
Funnel traps (larvae/m^2)	Sulphur 2005 [a]	S0			21.0	45.0	58.6	79.6	21.0	1.56	227
		S150			0.00	0.00	30.0	149	52.6	1.56	240
		RIR %			**-100**	**-100 ****	**-49**	**+87 ***	**+150 ***	**0**	**6**
Plant dissection (larvae/plant)		S0			12.4						
		S150			4.58						
		RIR %			**-63 ****						
Funnel traps (larvae/m^2)	Nitrogen 2005 [b]	N100			9.00	19.6	40.6	127	31.6	3.00	233
		N200			12.0	25.6	48.0	101	42.0	0.00	234
		RIR %			**+33**	**+31**	**+19**	**-21**	**+33**	**-100**	**+1**
Plant dissection (larvae/plant)		N100			7.43						
		N200			9.54						
		RIR %			**+28**						
Funnel traps (larvae/m^2)	Cultivar 2004 [c] Lipton Bristol	Lipton	25.6	114	31.6	70.6	57.0	18.0	1.56	3.00	322
		Bristol	15.0	117	55.6	49.6	52.6	16.6	4.56	3.00	313
		RIR %	**-41**	**+3**	**+76**	**-30**	**-8**	**-8**	**+200**	**0**	**-2**
Plant dissection (larvae/plant)		Lipton		4.75							
		Bristol		7.50							
		RIR %		**+58**							

Relative changes in the occurrence of *Ceutorhynchus obstrictus* larvae (%) for: a: S-fertilised plant compared with control (without S), b- Bristol cultivars compared with Lipton.*: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.25: Effect of S-fertilisation on the numbers of larvae of *Ceutorhynchus obstrictus* caught by funnel traps during different plant growth stages in 2004.

BBCH-scale	Sampling date	Cultivars	No. of larvae per trap relation to S-fertilisation (kg ha^{-1})	
			0	150
72	31.05.04	Lipton	1.75 a	2.50 a
		Bristol	1.25 a	1.25 a
73	06.06.04	Lipton	9.25 a	9.75 a
		Bristol	12.0 a	7.50 a
75	14.06.04	Lipton	13.0 a	2.25 a
		Bristol	6.25 a	3.00 a
76	21.06.04	Lipton	4.75 a	7.00 a
		Bristol	5.50 a	2.75 a
78	28.06.04	Lipton	2.50 a	7.00 a
		Bristol	4.50 a	4.25 a
whole-season		Lipton	23.0 a	30.5 a
		Bristol	31.3 a	21.0 a

No significant differences between treatments was found by U-test at 0.05 levels, n= 4.

Table A.26: Influence of S- and N-fertilisation on pods infected by larvae of *Ceutorhynchus obstrictus* at development of pod (BBCH 75), 06.06.2005.

Parameters	N-fertilisation (kg ha^{-1})	S-fertilisation (kg ha^{-1})	
		0	150
No. of *C. obstrictus* larvae per plant in main raceme	100	2.20 a A	0.30 b A
	200	5.13 a A	1.50 a A
No. of *C. obstrictus* larvae per plant in second raceme	100	9.54 a A	3.34 a A
	200	8.11 a A	4.14 a A
% of infected pods in main raceme	100	17.8 a A	18.4 a A
	200	27.0 a A	18.0 a A
% of infected pods in second raceme	100	27.6 a A	6.60 a A
	200	18.6 a A	10.2 a A

Mean values followed by different letters indicate significant differences by U-test at 0.05 level. n = 40. Uppercase letters are related to nitrogen application while lowercase letters are related to sulphur application.

Table A.27: The number of adults and the relative infection rate (RIR) of *Dasineura brassicae* collected by different methods in relation to S- and N-application and cultivar of oilseed rape at different growth stages (%).

Trapping method	Factor		Relative changes in the occurrence of adults of *Dasineura brassicae* at different growth stages (BBCH) relative to control (%)																Whole season
			62	64	65	66	69	72	75	76	77	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0		7.63	14.6	7.88	0.125		34.1						0.13				64.6
		S150		8.88	17.4	10.13	0.125		32.9						0.13				69.8
		RIR %		**+16**	**+19**	**+29**	**0**		**-4**						**0**				**+8**
Suction trap (adults/ 20 plants)		S0		5.0	13.3	4.13	5.38	0.38	42.5	19.3		0.38	0.38		1.25				91.9
		S150		6.0	16.5	2.38	4.62	0.88	42.0	37.1		1.88	0.25		1.75				113
		RIR %		**+20**	**+25**	**-42**	**-14**	**+133**	**-1**	**+93***		**+400****	**-33**		**+40**				**+23**
Emergence traps (adults/ m²)		S0							468	516		143	22.0	4500	2.52	0.52	1.00		1160
		S150							448	700		185	28.5	2.00	5.52	0.52	0.52		1372
		RIR %							**-4**	**+36**		**+29**	**+30**	**-56**	**+120**	**+100**	**-50**		**+18**
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2005)	S0	1.0	3.25		0.88			2.25	88.3		8.75	1.13	0.50	5.75				112
		S150	0.88	5.25		3.38			0.50	5.50		8.50	2.63	6.38	7.88				40.9
		RIR %	**-13**	**+62**		**+286***			**-78***	**-94*****		**-3**	**+133**	**+1175***	**+37**				**-63*****
Suction trap (adults/ 20 plants)		S0		2.25		2.0		0.38		1.88	59.1	12.6		0.75					79.0
		S150		0.13		1.25		0.13		0.25	2.63	37.5		9.63					51.5
		RIR %		**-94****		**-38**		**-67**		**-87**	**-96*****	**+197***		**+1183***					**-35**
Emergence traps (adults/ m²)		S0											124	27.52	102	97.2	11.0	4.00	366
		S150											99.2	51.2	228	464	26.0	1.52	868
		RIR %											**-20**	**+85**	**+122**	**+378**	**+136**	**-63**	**+137**

Table A.27: continued.

Trapping method	Factor		Relative changes in the occurrence of adults of *Dasineura brassicae* at different growth stages (BBCH) relative to control (%)																Whole season
			62	64	65	66	69	72	75	76	77	78	81	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Nitrogen [b] (2005)	N100	1.0	3.75		3.0			2.0	41.8		7.63	2.25	1	3.63				66.0
		N200	0.88	4.75		1.25			0.75	52.0		9.63	1.50	5.88	10				86.6
		RIR %	**-13**	**+27**		**-58**			**-63**	**+25**		**+26**	**-33**	**+488**	**+176***				**+31**
Suction trap (adults/ 20 plants)		N100		1.63		2.38		0.13		1.75	35.5	27.3		3.13					71.8
		N200		0.75		0.88		0.38		0.38	36.3	22.9		7.25					58.8
		RIR %		**-54**		**-63***		**+200**		**-79**	**-26**	**-16**		**+132**					**-18**
Emergence traps (adults/m²)		N100											106	42.0	130	81.6	6.00	3.52	368
		N200											118	36.5	200	480	31.0	2.00	868
		RIR %											**+11**	**-13**	**+54**	**+488**	**+417***	**-43**	**+135**
Sweep net (adults/ 40 sweeps)	Cultivar [c] (2004)	Lipton		7.75	16.3	8.13			31.1				0.125						63.4
		Bristol		8.75	15.8	9.88			35.9				0						71.0
		RIR %		**+13**	**-3**	**+22**			**+15**				**-100**						**+12**
Suction trap (adults/ 20 plants)		Lipton		4.0	16.0	3.38	2.38	0.38	38.3	27.6		0.75	0.38		1.50				94.1
		Bristol		6.75	13.8	3.13	7.63	0.88	46.3	28.8		1.50	0.25		1.50				110
		RIR %		**+69**	**-14**	**-7**	**+221***	**+133**	**+21**	**+4**		**+100**	**-33**		**0**				**+17**
Emergence traps (adults/m²)		Lipton							436	724		174	25.0	4.00	2.52	1.52	1.00		1368
		Bristol							480	492		154	25.52	2.52	5.52	0.00	0.52		1160
		RIR %							**+10**	**-32**		**-11**	**+2**	**-38**	**+120**	**-100**	**-50**		**-15**

Relative changes in the occurrence of *Dasineura brassicae* adults (%) for: a: S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by T-test.

Table A.28: Relative infection rate of larvae of *Dasineura brassicae* in relation to S- and N-application and cultivar of oilseed rape at different growth stages (%).

Trapping method	Factor		Relative changes in the occurrence of larvae of *Dasineura brassicae* at different growth stages (BBCH) relative to control (%)											Whole season
			67	71	72	73	75	76	78	81	83	86	89	
Funnel traps (larvae/ m^2)	Sulphur [a] (2004)	S0	3.00	640	726	966	229	16.6	2040	2736	1320	235.2		8916
		S150	5.16	583	888	1272	191	113	3936	3708	1752	400.8		12828
		RIR %	**+71**	**-9**	**+22**	**+31**	**-17**	**+582 ***	**+93 ***	**+35**	**+32**	**+70**		**+44 ***
Plant dissection (larvae/ plant)		S0				7.2								
		S150				15.9								
		RIR %				**121 ***								
Sweep net (larvae/ 40 sweeps)	Sulphur [a] (2005)	S0							0.75	32.0	39.1	42.9	5.38	120
		S150							0.00	1.88	8.63	6.75	5.88	23
		RIR %							**-100 ***	**-94 ****	**-78 ****	**-84 ***	**+9**	**-81 *****
Funnel traps (larvae/ m^2)		S0	10.6	2460	1620	269	246	3480	5232	756	129.6	433.2		14616
		S150	61.6	372	547	119	96	1121	6420	1572	169.2	306		10776
		RIR %	**+486 ***	**-85 ****	**-66 ****	**-56**	**-61 ***	**-68 ****	**+23**	**+108 ****	**+31**	**-30**		**-26 ***
Plant dissection (larvae/ plant)		S0					23.9							
		S150					8.21							
		RIR %					**-66 ****							

Table A.28: cntineued

Trapping methods	Factors		Relative changes in the occurrence of larvae of *Dasineura brassicae* at different growth stages (BBCH) relative to control (%)											Whole season
			67	71	72	73	75	76	78	81	83	86	89	
Sweep net (larvae/40 sweeps)	Nitrogen [b] (2005)	N100							0.50	11.5	28.0	30.4	4.75	75.1
		N200							0.25	22.4	19.8	19.3	6.50	68.1
		RIR %							**-50**	**+95**	**-29**	**-37**	**+37**	**-9**
Funnel traps (larvae/m^2)		N100	39		1145	894	127	161	2400	6864	1284	113	438	13452
		N200	33		1680	1272	259	181	2196	4788	1048	186	299	11940
		RIR %	**-15**		**+47**	**+42**	**+104**	**+13**	**-9**	**-30**	**-18**	**+65**	**-32**	**-11**
Plant dissection (larvae/plant)		N100					17.7							
		N200					19.4							
		RIR %					**+10**							
Funnel traps (larvae/m^2)	Cultivar [c] (2004)	Lipton	6.00	349	743	764	180	81	2976	4152	1992	420		11664
		Bristol	1.68	874	871	1464	240	48	2976	2292	1078	216		10068
		RIR %	**-71**	**+150 ***	**+17**	**+92**	**+33**	**-41**	**0**	**-45 ****	**-46 ***	**-49**		**-14**
Plant dissection (larvae/plant)		Lipton				10.3								
		Bristol				12.7								
		RIR %				**+23**								

Relative changes in the occurrence of *Dasineura brassicae* larvae (%) for S-fertilised plant compared with control (without S), high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level ; ***: Significant at 0.001 by T-test.

Table A.29: The number of adults and the relative infection rate (RIR) of *Phyllotretra* spp. collected by different methods in relation to S-fertilisation and cultivar of oilseed rape at different growth stages (%) for 2004.

Trapping method	Factor		Relative changes in the occurrence of adults of *Phyllotretra* spp. at different growth stages (BBCH) relative to control (%)																Whole season
			61	63	64	65	66	69	72	75	76	79	82	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	S0	9.38	12.6	4.0	12.0	1.13		0.50	0.63	1.37	1.13	0.50		0.13				43.4
		S150	11.9	17.7	3.5	11.2	1.75		0.63	0.50	0.38	2.0	0.0		0.63				50.1
		RIR %	**+27**	**+40**	**-13**	**-6**	**+56**		**+25**	**-20**	**-73 ***	**+78**	**-100**		**+400**				**+16**
Suction trap (adults/ 20 plants)		S0			2.50	1.88	0.50	0.63	1.38			0.25							7.50
		S150			2.00	1.63	1.25	0.63	1.63			0.0							7.88
		RIR %			**-20**	**-13**	**+150**	**0**	**+18**			**-100**							**+5**
Beating tray (adults/ 20 plants)		S0			1.13		0.38	2.38											3.88
		S150			0.75		0.50	0.13											1.34
		RIR %			**-33**		**+33**	**-95**											**-65**
Emergence traps (adults/ m^2)		S0							15.0		3.00	4.52	11.0	10.0	10.0	2.00	1.52	0.52	57.6
		S150							21.0		12.5	16.0	17.6	39.52	26.0	16.5	6.52	4.00	160
		RIR %							**+40**		**+317**	**+256**	**+64**	**+295**	**+160**	**+725 ***	**+333**	**+700**	**+178**

Table A.29: continued.

Trapping method	Factor		Relative changes in the occurrence of adults of *Phyllotretra* spp. at different growth stages (BBCH) relative to control (%)																Whole season
			61	63	64	65	66	69	72	75	76	79	82	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Cultivar[b]	Lipton	11.3	18.4	2.63	9.75	1.00		0.38	0.38	0.75	1.38	0.50		0.13				46.5
		Bristol	10.0	11.9	4.88	13.5	1.88		0.75	0.75	1.00	1.75	0.0		0.63				47.1
		RIR %	**-11**	**-35**	**+86 ****	**+38**	**+88**		**+100**	**+100**	**+33**	**+27**	**-100**		**+400**				**+1**
Suction trap (adults/ 20 plants)		Lipton			1.88	1.75	0.88	0.88		1.0	0.38	0.13	0.13						7.0
		Bristol			2.63	1.75	0.88	0.38		2.0	0.38	0.13	0.133						8.38
		RIR %			**+40**	**0**	**0**	**-57**		**+100**	**0**	**0**	**0**						**+20**
Beating tray (adults/ 20 plants)		Lipton			0.5		0.25	0.50											1.25
		Bristol			1.38		0.63	2.00											4
		RIR %			**+175**		**+150**	**+300**											**+220**
Emergence traps (adults/ m^2)		Lipton								26.0	9.00	16.0	18.0	36.0	22.5	12.0	4.52	1.00	145
		Bristol								10.4	6.52	4.52	11.0	13.5	13.5	6.52	3.52	3.52	72.4
		RIR %								**-62**	**-28**	**-72**	**-39**	**-63**	**-40**	**-46**	**-22**	**+250**	**-50**

Relative changes in the occurrence of *Phyllotretra* spp. adults (%) for a: S-fertilised plant compared with control (without S), b- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by T-test

Table A.30: Relative infection rate of adults of *Delia radicum* relative to S- and N-application and cultivars of oilseed rape at different growth stages.

Trapping method	Factors		Relative changes in the occurrence adults of cabbage root fly (*Delia radicum*) at different growth stages (BBCH) relative to control (%)																		Whole season
			30	53	61	63	64	65	66	69	71	75	76	78	81	82	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur 2004 [a]	S0			4.00	8.25	5.00	7.25	13.0		8.38	20.8	6.63	10.5		2.50					85.8
		S150			4.63	9.71	3.50	7.75	13.0		8.50	26.9	7.13	11.8		1.8					94.1
		RIR %			**+16**	**+18**	**-30**	**+7**	**0**		**+1**	**+30**	**+8**	**+12**		**-25**					**+10**
Suction trap (adults/ 20 plants)		S0					0.25	0.13	1.63	0.25	0.13	1.00	0.75	0.13	0.75						4.88
		S150					0.25	0.38	1.13	0.38	0.38	1.88	1.00	0.50	0.25						6.50
		RIR %					**0**	**+200**	**-31**	**+50**	**+200**	**+88**	**+33**	**+300**	**-67**						**+33**
Emergence traps (adults/ m^2)		S0										23.0	46.0	27.0	32.0	12.5	15.5	7.20	3.52	3.52	164
		S150										51.2	93.2	38.0	31.0	14.0	10.5	8.52	7.52	5.56	248
		RIR %										**+122**	**102**	**+41**	**-3**	**+12**	**-32**	**+21**	**+114**	**+57**	**+51**
Sweep net (adults/ 40 sweeps)	Sulphur 2005 [a]	S0	0.38	7.63		10.0	13.0		9.25		6.3	17.1	7.13	5.88	6.88	4.88	0.75			0.13	90.3
		S150	0.13	3.38		6.13	6.50		11.8		1.6	2.63	5.13	3.75	11.8	5.13	0.88			0.38	59.6
		RIR %	**-67**	**-56****		**-39**	**-50****		**+27**		**-75****	**-85*****	**-28**	**-36**	**+71***	**+5**	**+17**			**+200**	**-34*****
Suction trap (adults/ 20 plants)		S0																			4.00
		S150																			0.63
		RIR %																			**-84****
Emergence traps (adults/ m^2)		S0													17.7	15.0	10.0	8.00	12.0	54.4	128
		S150													15.5	8.00	6.00	24.0	8.00	18.3	93.2
		RIR %													**-13**	**-47***	**-40**	**+200**	**-33**	**-66****	**-27**

Table A.30: continued

Trapping method	Factors		Relative changes in the occurrence adults of cabbage root fly (*Delia radicum*) at different growth stages (BBCH) relative to control (%)																		Whole season
			3	53	61	63	64	65	66	69	71	75	76	78	81	82	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Nitrogen [b] (2005)	N100	0.13	0.63		8.25			9.63		11.8	3.38	8.13	2.63	6.00	9.50	5.38		0.50	0.13	72.6
		N200	0.38	0.88		7.88			9.88		9.25	0.50	11.6	9.63	3.63	9.13	4.63		1.13	0.38	77.3
		RIR %	**+200**	**+40**		**-5**			**+3**		**-21**	**+33**	**+43**	**267*****	**-40**	**-4**	**-14**		**+125**	**+200**	**+6**
Suction trap (adults/ 20 plants)		S0																			2.13
		S150																			2.50
		RIR %																			**+18**
Emergence traps (adults/ m^2)		S0													10.0	10.5	6.52	11.5	24.7		100
		S150													24.0	12.5	9.20	17.5	45.2		131
		RIR %													**140***	**+19**	**+46**	**+52**	**+82**		**+30**
Sweep net (adults/ 40 sweeps)	Cultivar [c] (2004)	Li pton			4.50	8.50	4.38	6.00	12.5		8.25	23.0	5.13	10.8		1.75		0.38			85.31
		Bristol			4.14	9.43	4.13	9.00	13.5		8.63	24.6	8.63	11.5		2.63		0.25			94.8
		RIR %			**-8**	**+11**	**-6**	**+50**	**+8**		**+5**	**+7**	**+68***	**+7**		**+50**		**-33**			**+11**
Suction trap (adults/ 20 plants)		Lipton					0.13	1.50		0.38	0.25	2.14	1.38	0.38	0.38		0.25				6.50
		Bristol					0.38	1.25		0.25	0.25	0.88	0.38	0.25	0.63		0.13				4.88
		RIR %					**+200**	**-17**		**-33**	**0**	**-59**	**-73**	**-33**	**+67**		**-50**				**-25**
Emergence traps (adults/ m^2)		Lipton										47.6	87.6	43.2	38.0		11.2	5.20	8.00	7.00	252
		Bristol										26.5	51.2	22.0	24.0		14.8	10.5	3.00	2.00	159
		RIR %										**-44**	**-42**	**-49**	**-37**		**+35**	**+110**	**-63**	**-71**	**-37**

Relative changes in the occurrence of *Delia radicum* adults (%) for a: S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by T-test.

Table A.31: Average number of adults of *Delia florilega* caught by sweep net in relation to S- and N-fertilisation and cultivar of oilseed rape over the whole-season in 2004 and 2005.

Variable	**No. of adults per 40 sweeps in relation to S-fertilisation (kg ha^{-1})**	
	0	150
Lipton (2004)	2.3 a A	2 a A
Bristol (2004)	3.3 a A	2 a A
N100 (2005)	5.5 a B	3.3 a B
N200 (2005)	7.5 a B	5.3 a B

No significant differences between treatment was found by U-test at 0.05 level. Uppercase letters A-A are related to cultivar of oilseed rape, uppercase letters B-B are related to nitrogen application while lowercase letters are related to sulphur application. n = 4.

Table A.32: Relative infection rate of adults of *Scaptomyza flava* relative to S- and N-application and cultivars of oilseed rape at different growth stages (%).

Trapping method	Factors		Relative changes in the occurrence adults of *Scaptomyza flava* at different growth stages (BBCH) relative to control (%)																		Whole season
			17	19	30	53	61	63	64	65	66	69	72	75	76	79	81	83	86	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0					1.43	0.63						1.75	0.25				0.25		4.13
		S150					2.13	0.88						2.88	0.63				0.25		7
		RIR %					**+49**	**+40**						**+64**	**+150**				**0**		**+70**
Suction trap (adults/ 20 plants)		S0							0.5	1.25	0.13	0.88	1.0	3.6	0.75	0.38	0.63		1.0		9.25
		S150							0.13	1.0	0.88	1.13	1.63	3.75	2	0.25	0.5		0.75		12
		RIR %							**-75**	**-20**	**+600 ***	**+29**	**+63**	**+2**	**+167**	**-33**	**-20**		**-25**		**+30**
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2005)	S0				0.25			0.88		0.13		0.38	2.38	0.5	0.13		0.13			4.75
		S150				0			0.13		0.38		0.13	1.38	2.63	0		0.25			5.5
		RIR %				**-100**			**-86**		**+200**		**-67**	**-42**	**+425 ****	**-100**		**+100**			**+16**
Suction trap (adults/ 20 plants)		S0	0.63	3.25	12.4	0.88			1		1	1.38	12.8	3.0			0.25		0.13		36.6
		S150	1.75	4.5	2.13	0			0.75		0.75	0.13	1.51	4.25			1.13		1.25		18.1
		RIR %	**+180**	**+38**	**-83 ****	**-100**			**-25**		**-25**	**-91 ****	**-88 *****	**+42**			**+350**		**+900**		**-51 ***
Emergence traps (adults/ m^2)		S0															2.52	1.52	0.52	22.5	56.4
		S150															0.00	4.52	25.5	27.5	63.6
		RIR %															**-100**	**+200**	**5000**	**+22**	**+12**

Table A.32: contineued.

Trapping method	Factors		Relative changes in the occurrence adults of *Scaptomyza flava* at different growth stages (BBCH) relative to control (%)																		Whole season
			17	19	30	53	61	63	64	65	66	69	72	75	76	79	81	83	86	99	
Sweep net (adults/ 40 sweeps)	Nitrogen [b] (2005)	N100				0.25			0.63		0.25	0.13	2.38	2.13			0.13	0.13			6.0
		N200				0			0.38		0.25	0.38	1.38	1.0			0.0	0.38			4.25
		RIR %				**-100**			**-40**		**0**	**+200**	**-42**	**-53**			**-100**	**+200**			**-29**
Suction trap (adults/ 20 plants)		N100	1.13	5.38	8.75	0.5			0.63		0.63	0.75	8.63	2.13	0.50						29.0
		N200	1.25	2.38	5.75	0.38			1.13		1.13	0.75	5.63	5.13	0.88						25.8
		RIR %	**+11**	**-56**	**-34**	**-25**			**+80**		**+80**	**0**	**-35**	**+141**	**+75**						**-11**
Emergence traps (adults/m²)		N100													0.52	2.52	0.52	1.52	9.00	2.52	16.5
		N200													2.00	23.5	0.52	14.0	41.2	16.5	104
		RIR %													**+300**	**+840**	**0**	**+833**	**356***	**+560**	**527***
Sweep net (adults/ 40 sweeps)	Cultivar [c] (2004)	Lipton					2.5	1						3.38	0.75		0.25		0.38		8.25
		Bristol					1	0.5						1.25	0.13		0.0		0.13		2.88
		RIR %					**-60***	**-50**						**-63**	**-83**		**-100**		**-67**		**-65****
Suction trap (adults/ 20 plants)		Lipton							0.38	1.0	0.25	0.63	1.25	4.85	2.13	0.25	0.25	0.88			11.3
		Bristol							0.63	1.25	0.38	1.38	1.38	2.57	0.63	0.38	0.88	0.88			10.0
		RIR %							**+67**	**+25**	**+50**	**+120**	**+10**	**-47**	**-71***	**+50**	**+250**	**0**			**-11**

Relative changes in the occurrence of Scaptomyza flava adults (%) for a: S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by T-test

Table A.33: Relative infection rate of adults of *Brevicoryne brassicae* in relation to S- and N-application and cultivars of oilseed rape at different growth stages (%) in 2004 and 2005.

Trapping method	Factors		Relative changes in the occurrence of *Brevicoryne brassicae* adults relative to S-fertilisation at different growth stages (%)												Whole season
			65	66	69	72	75	76	78	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0	2.88	2.88		0.75	8.25	5.13	12.9	14.0		0.50			47.3
		S150	3.50	3.50		0.38	14.4	15.1	21.3	6.00		0.63			64.8
		RIR %	**+22**	**+22**		**-50**	**+74**	**+195**	**+65**	**-57**		**+25**			**+37**
Suction trap (adults/ 20 plants)		S0	3.50	8.25	5.50	2.50	6.88	20.0	27.6	30.9		1.75			107
		S150	1.88	7.50	0.25	1.63	20.9	35.4	113	84.5		2.63			268
		RIR %	**-46**	**-9**	**-95**	**-35**	**204**	**+77**	**+309**	**174**		**+50**			**+150**
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2005)	S0		0.13		0.88	1.38	0.25	2.88	8.13	22.4	34.4		0.63	95.6
		S150		0.38		0.50	0.50	0.38	0.88	0.25	4.63	12.9		3.63	37.6
		RIR %		**200**		**-43**	**-64**	**+50**	**-70**	**-97 ****	**-79 ****	**-63**		**480 ***	**-61**
Suction trap (adults/ 20 plants)		S0						0.38	1.25		9.75				12.9
		S150						0.25	4.75		8.38				13.6
		RIR %						**-33**	**+280**		**-14**				**+6**
Emergence traps (adults/ m^2)		S0								4.52	2.52		1.50	3.00	12.0
		S150								8.00	3.00		40.4	18.5	86.0
		RIR %								**+78**	**+20**		**+2600**	**517**	**+617**

Table A.33: continued.

Trapping method	Factors		Relative changes in the occurrence of *Brevicoryne brassicae* adults relative to N-fertilisation and cultivars at different growth stages (%)												Whole season
			65	66	69	72	75	76	78	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Nitrogen [b] (2005)	N100		0.13		1.0	1.0	0.13	3.13	6.75	17.4	30.9		1.88	88.1
		N200		0.38		0.38	0.88	0.50	0.63	1.63	9.63	16.4		2.38	45.1
		RIR %		**200**		**-63**	**-13**	**+300**	**-80**	**-76**	**-45**	**-47**		**+27**	**-49**
Suction trap (adults/ 20 plants)		N100						0.63	3.38		12.4				17.6
		N200						0.0	2.63		5.75				8.88
		RIR %						**-100**	**-22**		**-54**				**-50**
Emergence traps (adults/ m²)		N100								4.52	0.52	0.52	2.00	2.52	10.0
		N200								8.00	5.00	14.5	40.0	19.0	88.0
		RIR %								**+78**	**900**	**+2800**	**+1900**	**660 ***	**+780**
Sweep net (adults/ 40 sweeps)	Cultivar [c] (2004)	Lipton	3.88	3.88		0.50	9.63	12.3	12.8	13.0		0.63			56.5
		Bristol	2.50	2.50		0.63	13.0	8.00	21.4	7.00		0.50			55.5
		RIR %	**-35**	**-35**		**+25**	**35**	**-35**	**+68**	**-46**		**-20**			**-2**
Suction trap (adults/ 20 plants)		Lipton	2.25	10.6	5.38	1.25	25.4	41.2	85.6	58.4		1.75			232
		Bristol	3.13	5.13	0.38	2.88	2.38	14.1	54.9	57.0		2.63			143
		RIR %	**+39**	**-52 ***	**-93**	**130**	**-91**	**-66**	**-36**	**-2**		**+50**			**-39**

Relative changes in the occurrence of *Brevicoryne brassicae* adults (%) for: a S-fertilised plant compared with control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level by T-test.

Table A.34: Relative infection rate with adults and larvae of *Staphylinidae* family in relation to S- and N-application and cultivars of oilseed rape at different growth stages (%) in 2004 and 2005

Trapping method	factors		Relative changes in the occurrence of *Staphylinidae* (adults and larvae) at different growth stages (BBCH) relative to control (%)																	Whole season
			53	64	66	67	71	74	75	76	78	79	81	82	83	86	89	97	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0		0.50	0.25		0.13			0.25		0.25		0.38		0.13				1.88
		S150		0.75	0.13		0.0			0.0		0.0		0.13		0.38				1.50
		RIR %		**+50**	**-50**		**-100**			**-100**		**-100**		**-67**		**+200**				**-20**
Emergence traps (adults/ m²)		S0						19.0		19.0	47.6		70.4		113	85.6	60.4	13.5	13.5	444
		S150						28.5		49.2	76.8		77.6		91.2	75.2	54.0	15.0	18.0	484
		RIR %						**+50**		**158****	**+62**		**+10**		**-20**	**-12**	**-11**	**+11**	**+33**	**+10**
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2005)	S0	0.38				0.50		0.38	0.38			0.13			0.13				1.88
		S150	0.0				0.25		0.25	0.63			0.0			0.13				2.38
		RIR %	**-100**				**-50**		**-33**	**+67**			**-100**			**0**				**+27**
Emergence traps (adults/ m²)		S0											186		87.6	110	73.6	30.0	34.0	540
		S150											97.6		74.4	64.4	51.2	16.5	45.6	374
		RIR %											**-47 ***		**-15**	**-41**	**-31**	**-45**	**+34**	**-31**
Funnel traps (Larvae/ m²)		S0				1.56		7.56	15.0	116	228		94.6		7.56					469
		S150				0.0		12.0	6.0	19.6	197		88.6		25.6					348
		RIR %				**-100**		**+60**	**-60**	**-83 ****	**-14**		**-6**		**+240**					**-26**

Table A.34: contieued.

Trapping method	factors		53	64	66	67	71	74	75	76	78	79	81	82	83	86	89	97	99	Whole season
			Relative changes in the occurrence of *Staphylinidae*(adults and larvae) at different growth stages (BBCH) relative to control (%)																	
Sweep net (adults/ 40 sweeps)	Nitrogen [b] (2005)	N100	0.25				0.13			0.38			0.13			0.13		0.13		1.13
		N200	0.13				0.63			0.63			0.00			0.13		0.88		3.13
		RIR %	**-50 ***				**+400**			**+67**			**-100**			**0**		**+600**		**+178 ***
Emergence traps (adults/ m^2)		N100											98.4		60.4	61.2	47.6	14.0	17	334
		N200											184		102	114	77.2	32.5	26	580
		RIR %											**+87 ***		**+68 ***	**+88 ***	**+62**	**+132**	**+53**	**+73 ***
Funnel traps (larvae/ m^2)		N100						15.0	10.6	91.6	228		108		21.0					474
		N200						4.56	10.6	43.6	197		75.0		12.0					343
		RIR %						**-70**	**0**	**-52**	**-14**		**-31**		**-43**					**-28**
Sweep net (adults/ 40 sweeps)	Cultivar [c] (2004)	Lipton		0.75	0.25		0.25		0.13	0.25		0.13								1.75
		Bristol		0.5	0.13		0		0.13	0.25		0.38								1.63
		RIR %		**-33**	**-50**		**-100**		**0**	**0**		**+200**								**-7**
Emergence traps (adults/ m^2)		Lipton							19.0	34.5	76.4		91.2		118	90.0	59.6	13.5	16.0	516
		Bristol							28.5	33.5	48.0		57.2		87.2	70.4	55.2	15.0	15.5	412
		RIR %							**+50**	**-3**	**-37 ***		**-37**		**-26**	**-22**	**-8**	**+11**	**-33**	**-21**

Relative changes in the occurrence of *Staphylinidae*(adults and larvae) (%) for: a S-fertilised plant compared to control (without S), b- high dose of N-fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.35: Relative infection rate with adults and larvae of *Tachyporus* genus in relation to S-application and cultivars of oilseed rape at different growth stages (%) in 2004 and 2005.

Trapping method	Factors		Relative changes in the occurrence of *Tachyporus (adults* and larvae) at different growth stages (BBCH) relative to control (%) Whole season															Whole season
			66	67	71	72	73	74	75	76	78	81	83	86	89	97	99	
Emergence traps (adults/ m²)	Sulphur [a] (2004)	S0						1.52		0.52		8.52	48.0	84.4	36.0	5.52	1.52	186
		S150						1.00		0		4.00	35.5	95.6	36.5	3.52	0.52	176
		RIR %						**-33**		**-100**		**-53**	**-26**	**+13**	**+1**	**-36**	**-67**	**-5**
Funnel traps (larvae/ m²)		S0	1.56		10.6	101	227		246	126	55.6	22.6	12.0	3.00				804
		S150	0.00		10.6	108	317		364	108	37.6	24.0	9.00	0.00				979
		RIR %	**-100**		**0**	**+7**	**+40**		**+48**	**-14**	**-32**	**+7**	**-25**	**-100**				**+22**
Emergence traps (adults/ m²)	Sulphur [a] (2005)	S0										13.5	70.0	69.6	34.0	3.52	4.00	198
		S150										2.5	19.0	15.0	7.00	1.52	0.52	45.6
		RIR %										**-81 ***	**-73 ****	**-78 ****	**-79*****	**-57**	**-88 ***	**-77*****
Funnel traps (larvae/ m²)		S0		3.00			118.6		131	499	311	73.6	6.00	1.56				1144
		S150		0.00			3.00		27.0	124	192	72.0	9.00	1.56				412
		RIR %		**-100**			**-97**		**-79 ***	**-75 ***	**-38**	**-2**	**50**	**0**				**-64 ***

Table A.35: continued.

Trapping method	Factors		Relative changes in the occurrence of *Tachyporus adults* and larvae)at different growth stages (BBCH) relative to control (%)															Whole season
			66	67	71	72	73	74	75	76	78	81	83	86	89	97	99	
Emergence traps (adults/ m^2)	Nitrogen [b] (2005)	N100										12.0	52.0	44.4	19.0	1.52	3.52	137
		N200										4.00	37.0	40.0	22.0	3.52	1.00	108
		RIR %										**-67**	**-29**	**-10**	**+16**	**133**	**-71**	**-21**
Funnel traps (larvae/ m^2)		N100			97.6				113	396	324	97.6	1.56					1013
		N200			24.0				45.0	227	179	48	13.6					541
		RIR %			**-75**				**-60**	**-43**	**-45**	**-51**	**+800**					**-47**
Sweep net (adults /40 sweeps)	Cultivar [c] (2004)	Lipton						0.38				1.63	12.3	25.3	9.5	1.0	0.13	50.1
		Bristol						0.25				1.5	8.63	19.8	8.63	1.25	0.38	40.5
		RIR %						**-33**				**-8**	**-30**	**-22**	**-9**	**+25**	**+200**	**-9**
Emergence traps (adults/ m^2)		Lipton	0.52		3.52	35.5	92.0	108	45.6			10.5	4.00	0.52				320
		Bristol	0.0		3.52	34.0	89.2	94.4	32.5			5.00	3.00	0.52				275
		RIR %	**-100**		**0**	**-4**	**-3**	**-13**	**-29**			**-52 ***	**-25**	**0**				**-14**

Relative changes in the occurrence of *Tachyporus* (adults and larvae (%) for: a S-fertilised plant compared to control (without S), b- high dose of N fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), c- Bristol cultivars compared with Lipton. *: Significant at 0.05 level; **: Significant at 0.01 level; ***: Significant at 0.001 level by U-test.

Table A.36: Relative infection rate of spider relative to S-application and cultivars of oilseed rape at different growth stages (%) in 2004.

Trapping method	Factors		Relative changes in the occurrence of spider adults at different growth stages (BBCH) relative to control (%)																	Whole season
			61	63	64	64	66	69	72	74	75	76	78	79	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a] (2004)	S0	0.13	1.00	0.25	1.50	2.75		3.75		7.50	1.00		1.63						19.5
		S150	0.75	1.25	1.25	0.63	2.13		4.00		5.88	1.13		1.25						18.3
		RIR %	**+500***	**+25**	**+400***	**-58**	**-23**		**+7**		**-22**	**+13**		**-23**						**-6**
Suction trap (adults/ 20 plants)		S0			0.50	0.50	0.50	1.00			1.50	1.00								6.25
		S150			0.13	0.88	0.75	0.14			2.43	0.25								5.13
		RIR %			**-75**	**+75**	**+50**	**-86**			**+62**	**-75***								**-18**
Beating tray (adults/ 20 plants)		S0			0.25		0.75	0.63												1.63
		S150			0.50		1.50	0.63												2.63
		RIR %			**+100**		**+100**	**0**												**+62**
Emergence traps (adults/ m^2)		S0								40.0		46			181	186	350	620	270	1904
		S150								38.1		62			120	256	374	360	104	1488
		RIR %								**-5**		**+30**			**-33**	**+38**	**+7**	**-42**	**-61****	**-22**

Table A.36: continued.

Trapping method	Factors		Relative changes in the occurrence of spider adults at different growth stages (BBCH) relative to control (%)																	Whole season
			61	63	64	64	66	69	72	74	75	76	78	79	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Cultivar[b] (2004)	Lipton	0.50	1.50	0.88	1.00	2.50		4.50		5.25	0.88		1.13						18.1
		Bristol	0.38	0.75	0.63	1.13	2.38		3.25		8.13	1.25		1.75						19.6
		RIR %	**-25**	**-50**	**-29**	**+13**	**-5**		**-28**		**+55**	**+43**		**+56**						**+8**
Suction trap (adults/ 20 plants)		Lipton			0.38	0.63	1.13	0.38	1.25		2.38	0.50								6.63
		Bristol			0.25	0.75	0.13	0.86	0.88		1.43	0.75								4.75
		RIR %			**-33**	**+20**	**-89***	**+129**	**-30**		**-40**	**+50**								**-28**
Beating tray (adults/ 20 plants)		Lipton			0.25		1.25	0.63												2.13
		Bristol			0.50		1.00	0.63												2.13
		RIR %			**+100**		**-20**	**0**												**0**
Emergence traps (adults/ m^2)		Lipton								11.5		15.0	11.5		30.0	42.0	58.0	134	39.0	388
		Bristol								8.00		9.0	15.0		45.2	68.4	123	110	31.5	456
		RIR %								**-30**		**-40**	**+30**		**+50**	**+63**	**+112**	**-18**	**-19**	**+18**

Relative changes in the occurrence o *spider*(%) for: a S fertilised plant compared to control (without S), b- high dose of N fertilised plant (200 kg N ha^{-1}) compared with low dose of N (100 kg N ha^{-1}), b- Bristol cultivars compared with Lipton. *: Significant at 0.05 level.

Table A.37: Relative infection rate of Thrips relative to S-application and cultivars of oilseed rape at different growth stages (%) in 2004.

Trapping method	Factors		Relative changes in the occurrence of *Thrips* adults at different growth stages (BBCH) relative to control (%)																	Whole season
			61	63	64	65	66	69	72	74	75	76	78	79	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Sulphur [a]	So	0.63	1.25	3.00	2.50	1.38		1.00		0.63	0.25		0.63						11.3
		S150	0.88	0.88	4.88	0.88	1.25		2.13		0.50	0.50		0.25						12.1
		RIR %	**+40**	**-30**	**+63**	**-65**	**-9**		**+113**		**-20**	**+100**		**-60**						**+8**
Suction trap (adults/ 20 plants)		So			2.75	1.13	1.50	1.25	1.63		1.38	0.63	0.38		0.50					11.1
		S150			2.13	1.38	2.38	1.00	1.63		0.25	1.38	0.25		0.38					10.8
		RIR %			**-23**	**+22**	**+58**	**-20**	**0**		**-82**	**+120**	**-33**		**-25**					**-3**
Beating tray (adults/ 20 plants)		So			1.25		1.00	1.75												4.00
		S150			1.75		0.75	1.38												3.88
		RIR %			**+40**		**-25**	**-21**												**-3**
Emergence traps (adults/ m^2)		So								4.00		3.52	7.00		4.52	4.00	4.52	1.52	2.52	36.5
		S150								3.00		2.00	4.00		2.00	5.00	2.52	1.00	5.00	24.5
		RIR %								**-25**		**-43**	**-43**		**-56**	**+25**	**-44**	**-33**	**-80**	**-33**

Table A.37: continued.

Trapping method	Factors		Relative changes in the occurrence of *Thrips* adults at different growth stages (BBCH) relative to control (%)																	Whole season
			61	63	64	65	66	69	72	74	75	76	78	79	81	83	86	89	99	
Sweep net (adults/ 40 sweeps)	Cultivar [b]	Lipton	0.75	1.25	4.88	1.50	0.75		1.88		0.38	0.50		0.38						12.3
		Bristol	0.75	0.88	3.00	1.88	1.88		1.25		0.75	0.25		0.50						11.1
		RIR %	**0**	**-30**	**-38**	**+25**	**+150**		**-33**		**+100**	**-50**		**+33**						**-9**
Suction trap (adults/ 20 plants)		Lipton			2.88	1.50	2.13	1.13	1.13											0.63
		Bristol			2.00	1.00	1.75	1.13	2.13											1.00
		RIR %			**-30**	**-33**	**-18**	**0**	**+89**											**-1**
Beating tray (adults/ 20 plants)		Lipton			1.75		0.50	1.63												3.88
		Bristol			1.25		1.25	1.50												4.00
		RIR %			**-29**		**+150**	**-8**												**+3**
Emergence traps (adults/ m^2)		Lipton								4.00		4.00	5.00		3.52	4.52	3.52	1.00	5.52	32.5
		Bristol								3.00		1.52	6.00		3.00	4.52	3.52	1.52	4.00	28.5
		RIR %								**-25**		**-63**	**+20**		**-14**	**0**	**0**	**+50**	**-27**	**-12**

Relative changes in the occurrence of *Thrips* (%) for: a S fertilised plant compared to control (without S), b- Bristol cultivars compared with Lipton

Appendix III

A1	A2	A2	A1	A2	A1	A2	A2	A1	A2	A1	A1	A2	A1	A2	A1
B1	B1	B1	B1	B1	B1	B2	B2	B2	B1	B2	B2	B2	B2	B2	B1
C2	C1	C2	C1	C2	C2	C1	C1	C1	C1	C1	C2	C2	C2	C2	C1
D1	D1	D2	D2	D1	D2	D2	D1	D1	D2	D2	D2	D1	D1	D2	D1
16	15	14	13	32	31	30	29	48	47	46	45	64	63	62	61

A2	A1	A2	A1	A1	A2	A1	A1	A2	A2	A1	A2	A1	A2	A1	A2
B2	B2	B1	B1	B2	B2	B2	B2	B1	B2	B1	B1	B1	B1	B1	B2
C2	C1	C1	C1	C2	C2	C1	C2	C1	C1	C2	C2	C2	C2	C1	C1
D1	D1	D2	D1	D1	D2	D2	D2	D1	D1	D1	D1	D2	D2	D2	D2
9	10	11	12	25	26	27	28	41	42	43	44	57	58	59	60

A2	A2	A2	A2	A2	A2	A2	A2	A1	A1	A1	A1	A1	A1	A1	A1
B2	B1	B2	B2	B1	B2	B1	B1	B1	B1	B2	B1	B2	B2	B1	B2
C1	C2	C2	C1	C1	C2	C2	C1	C1	C2	C2	C1	C1	C2	C2	C1
D2	D1	D2	D1	D1	D1	D2	D2	D2	D2	D1	D1	D1	D2	D1	D2
8	7	6	5	24	23	22	21	40	39	38	37	56	55	54	53

A1	A1	A1	A1	A1	A1	A1	A1	A2	A2	A2	A2	A2	A2	A2	A2
B2	B2	B2	B1	B2	B1	B1	B1	B2	B2	B2	B1	B2	B1	B1	B1
C2	C2	C1	C2	C1	C1	C2	C1	C2	C2	C1	C2	C1	C1	C2	C1
D2	D1	D2	D2	D1	D2	D1	D1	D2	D1	D2	D2	D1	D2	D1	D1
1	2	3	4	17	18	19	20	33	34	35	36	49	50	51	52

Fig. A.1: The distribution of traps in the field (season 2003/2004)

A1: Bristol, A2: Lipton

B1: 0 kg S ha^{-1}, B2: 150 kg S ha^{-1}

C1: 100 kg N ha^{-1}, C2: 150 kg Nha^{-1}

D1: without fungicide, D2: with fungicide

☼: Emergence traps, ⧗: Funnel traps

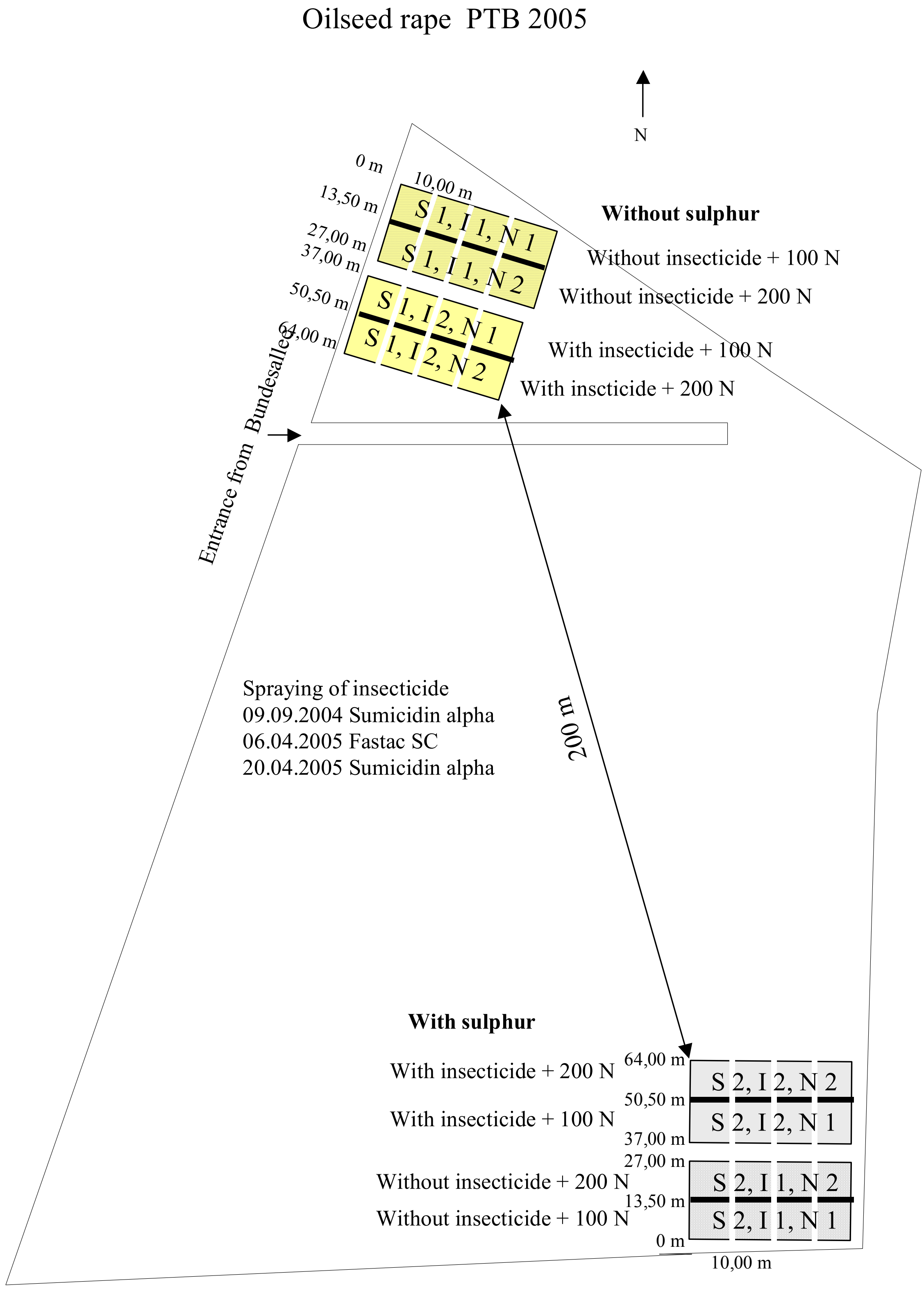


Fig. A.2: The distribution of plots in the PTB field (season 2004/2005)

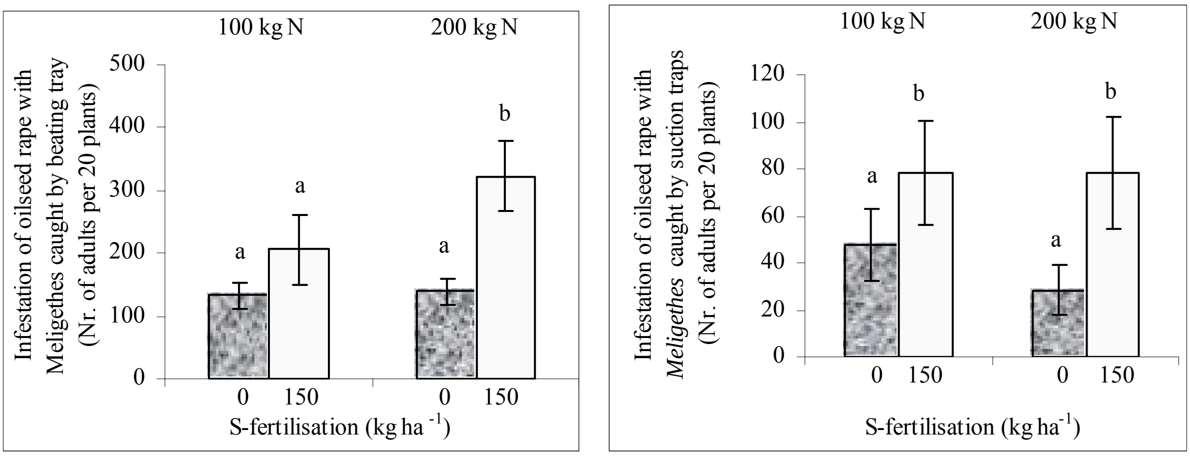


Fig. A.3: Number of adults of *Meligethes* spp. caught by beating tray and suction trap at full flowering (BBCH 66) relation to S- and N-fertilisation (different letters denote significant differences between treatments at the 0.02 level by the U-test).

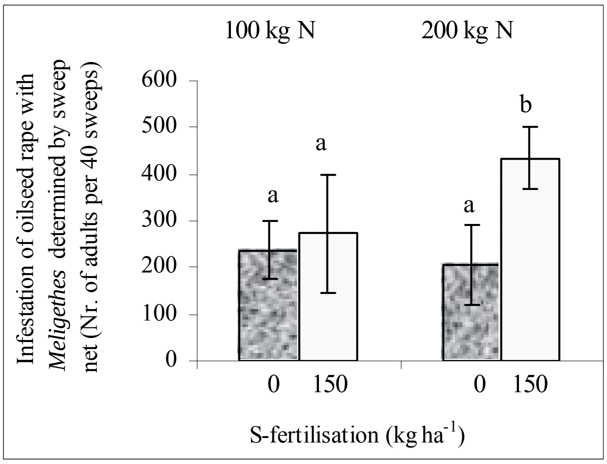
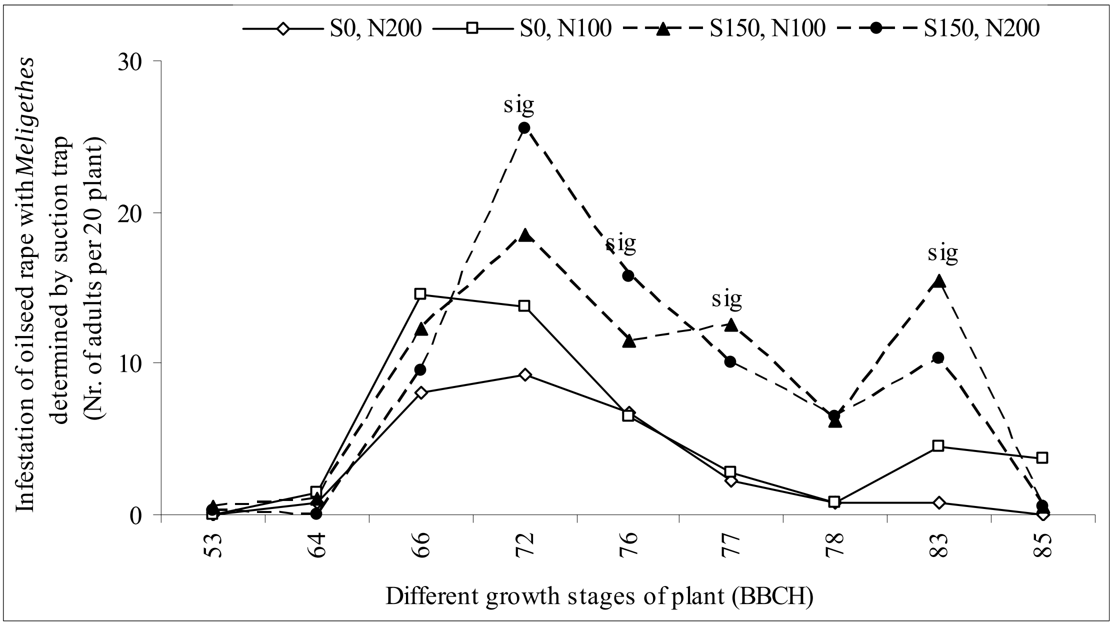


Fig. A.4: Influence of S-fertilisation on the *Meligethes* spp. adults caught by a sweep net in the season 2004/2005 (different letters denote significant differences between treatments at the 0.02 level by the U-test).



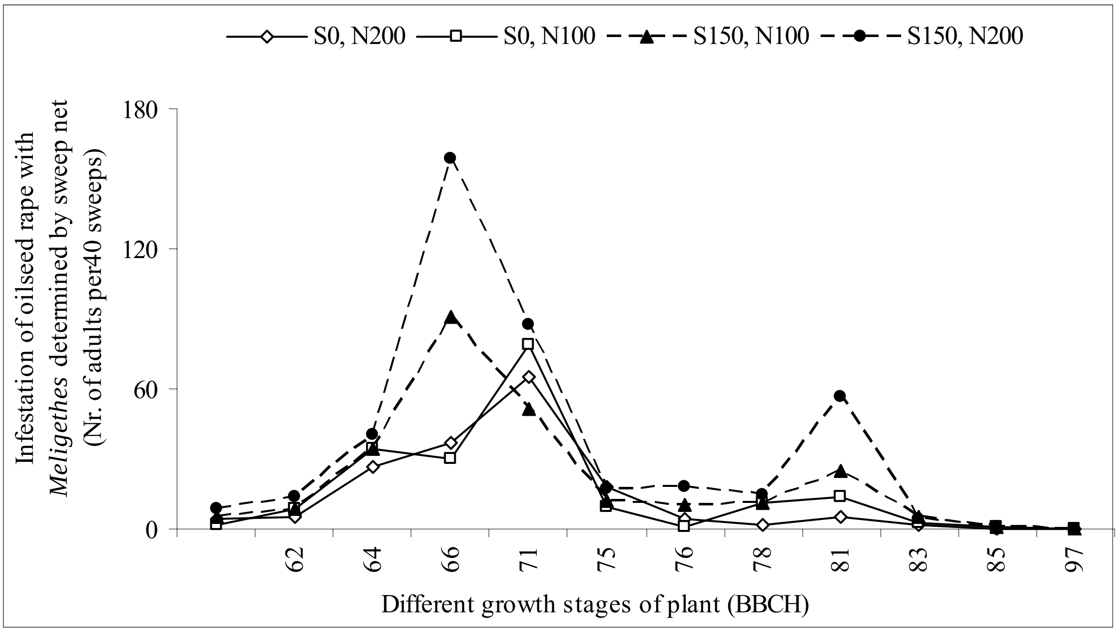


Fig. A.5: Response of *Meligethes* spp. adults to S-fertilisaton during different growth stages of plant (insects were collected by sweep net and suction trap) (different letters denote significant differences between treatments at the 0.02 level by the U-test) (S0 plots without S-application, S150 plots which received 150 kg S ha^{-1}, N100 plants which received 100 kg N ha^{-1} while N 200 plants that fertilised with 200 kg N ha^{-1}).

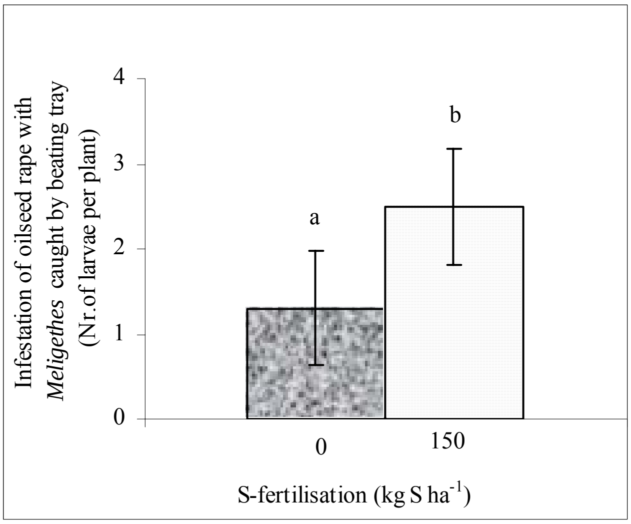


Fig. A.6: Numbers of *Meligethes* larvae in oilseed rape (Lipton) in relation to S-fertilisation (insects were caught at BBCH 66 by beating tray) (data from 2004) (different letters denote significant differences between treatments at the 0.05 level by the U-test).

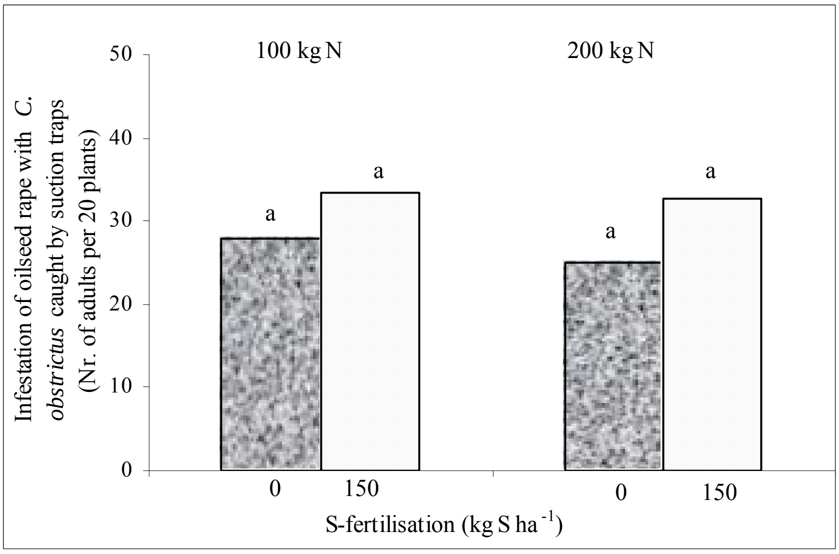


Fig. A.7: Effect of S-fertilisation on the quantity numbers of adults of *Ceutorhynchus obstrictus* (insects were collected by suction trap in season 2004/2005) (different letters denote to significant differences between treatments at the 0.05 level by the U-test).

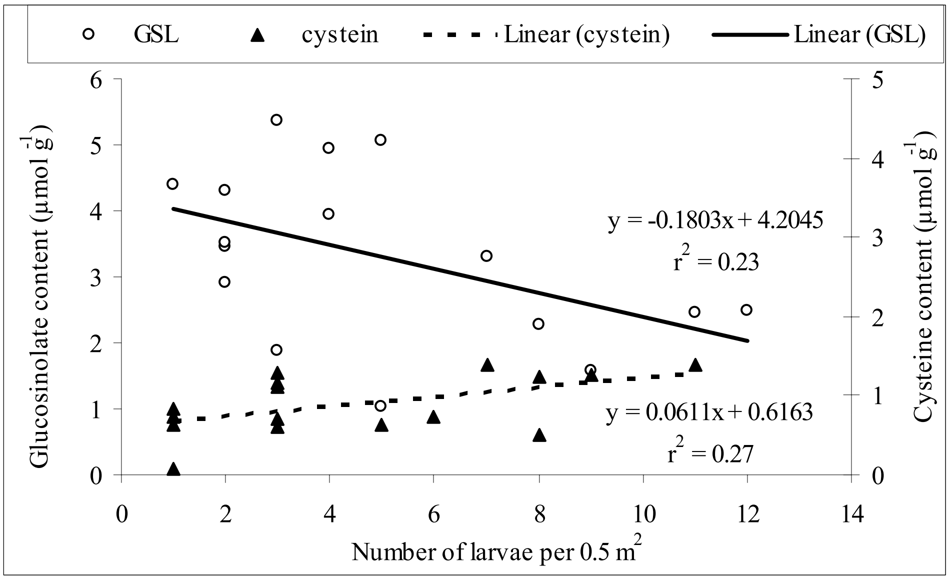


Fig. A.8: The relationship between S-compounds (glucosinolates, cysteine) and occurrence of pods with *Ceutorhynchus obstrictus* larvae in early pod development.